STUDIES IN IMMUNITY.

More Especially With Reference To Some Of The Reactions Of Complement.

AND

To The Seat Of Origin Of Complement And Immune Body.

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I beg to submit this work as a thesis for the M.D. Degree. A piece of work, on the subject of this research, has been already published, in the Journal of Pathology and Bacteriology; but as it is of some importance to the main argument, I would ask permission to present it also. This work is contained in a reprint, from the above-mentioned Journal bound into the Thesis.

I N T R O D U C T I O N.

Two years ago Dr.Ritchie asked me to investigate the histological changes that take place in the organs of the Rabbit, when injected intravenously with washed hens' corpuscles. The normal rabbits serum, in vitro, is sometimes lytic to hens' corpuscles, sometimes not. Twelve rabbits were injected intravenously each with 2cc. washed hens' corpuscles and killed, at various times after injection from 10 minutes up to 48 hrs. The organs of the animals were at once put into various fixatives, - alcohol, saturated $HgCl_2$ and 5% formalin. The chief phenomenon, observed after injection, was the sudden appearance of Haemoglobin in the urine, as early as 20 minutes after injection (in the case of those rabbits with serum lytic in vitro). This haemoglobinuria had generally passed off by the end of 5 hours. No hens' corpuscles were to be seen in the urine and no rabbits' corpuscles were seen either, thus excluding haemorrhages in the kidney.

The organs examined microscopically were the liver, spleen, kidney and lung; they were examined chiefly for the presence of hens' corpuscles and the changes in them, for haemorrhages, and for phagocytosis: no/

No hens corpuscles and no haemorrhages were seen in any of the kidneys examined. In the spleens, the hens corpuscles were seen in some cases up to 1 hour: after this they could not be detected. No phagocytosis of the corpuscles was to be seen in the spleen. In the lung, as in the kidney, the corpuscles were not detectable. The absence of the corpuscles in the kidney and lung, as well as their early disappearance from the spleen, seems to be accounted for by the appearances in the liver. Here from the very first, the corpuscles were seen in large numbers, continued to be present in large numbers up to at least 5 hours, when no other observation was made up to 24 hrs, when they could not be found. In the liver too, the corpuscles were seen in solid plugs agglutinated together, sometimes with the cell membranes gone and the whole appearing like a large multinucleated Haemoglobin containing cell. As compared with the other tissues examined, then, the hens corpuscles were very easy to find in the liver, and could be found there for a much longer time after injection than in any other organ. And again, in the liver, were to be seen a few instances of phagocytosis (by Kupfer's cells and liver cells),- a phenomenon which was not observed in any of the other organs. The deduction, that/

that one, at first sight, feels inclined to make from the long continuance in the liver and rapid disappearance from the spleen (and other organs), is that the spleen &c, are the seat of the lytic action and that the liver on the other hand has no such action. But from the rapidity of the lysis (in about 20 minutes Hb. appeared in the urine), the absence of marked phagocytosis in the liver, and its total absence in the spleen, and from other facts, which will accumulate later, I consider neither the spleen, nor the liver to be the seat of the lysis: but that it takes place in the general blood stream, and that the accumulation of the hens' corpuscles in the liver, is merely an expression of the mechanics of the circulation. The liver is a spongework of cells, and can expand to contain an enormous amount of blood: and from the microscopical examination above, it wouldappear that it can collect foreign bodies from the blood stream, and can store them up in its capillaries, even as plugs, without danger of stoppage of the circulation. The low blood pressure in the portal vessels, and the large blood content, materially, assist the liver in its filtering function, and permit, more than in any other organ, stagnation and opportunity for the fixed cells of the tissue to act on foreign solid particles. Again the fineness/

fineness of the capillary endothelium.- so fine and so interrupted.that some doubt its existence as an endothelium, - and the presence of Kupfers phagocytic cells.all favour this intimate association between the foreign particles and the liver parenchyma. From these considerations,I thought it desirable, while investigating in the following research various problems in immunity,to focus on the possibility of the liver being the seat of formation of Immune body and complement. Unfortunately a direct investigation of this is unattainable, although some work of this nature.as will be mentioned later, has been done. Mammals, with the liver cut out of the circulation, die in a few hours, birds in a few days, from the profound disturbance of metabolism, acidosis, etc., that takes place. In the case of the complement, as will be seen later, it has been found by Nolf that cutting out the liver from the circulation in the rabbit practically destroys the complement in the blood: but the metabolic changes,by affecting some other organ, the true seat,let us say of the formation of complement,might produce this effect altogether independent of the liver having anything to do with the production of complement. In the case of the Immune body, this is not formed in less than 3 days even/

even by the quickest method of production that of intravenous injection. Mammals do not live for more than a day or so with an Eck's fistula and for them, and for birds, with the vein of Jacobson ligatured, the same criticism holds, in immune body production as for complement production - that the metabolic changes, incident on extirpation of the liver, might of themselves cause the failure of production of the immune body. As a matter of fact, the experiment (Ecks fistula &c) is very difficult indeed to perform, and with such a valid objection to the methods at the outset, I did not think it worth while to attack the question in this way. Indeed I think - and this tells for my contention later, that the liver may be the seat of formation of complement and immune body - that the liver is of such fundamental importance to ultimate metabolism that any interference with the functions of the organ, by its removal from the circulation, in the hope of taking away at the same time some pure function and thus gaining absolute proof of the liver being the seat of that function, is destined to failure, either by the rapid death of the animal, or by a total upset of metabolism, leading to conditions from which no honest conclusions can be drawn/

drawn. Recognising these facts the only other method left is that of exclusion and circumstantial evidence and I shall treat the subject in 3 sections in this manner giving:-

1st. The evidence for other organs than the liver being the seat of formation of immune body with some indirect circumstantial evidence for the liver being the seat.

2nd. The evidence for other organs than the liver being the seat of formation of complement with some indirect evidence for the liver being the seat.

3rd. The more direct evidence for the liver being the seat of formation of both immune body and complement.

S E C T I O N I.

EVIDENCE IF ANY FOR OTHER ORGANS THAN THE LIVER BEING THE SEAT OF FORMATION OF IMMUNE BODY AND SOME INDIRECT EVIDENCE FOR THE LIVER BEING SUCH A SEAT.

L I T E R A T U R E.

The literature of this subject is very extensive and very confusing. I shall give first the literature dealing with the alexins, protective substances, agglutinins, precipitins, and haemo and bacteriolysins, with a critical survey of this literature: I shall then give the literature dealing with feeding experiments, which have a bearing on the indirect evidence of this section, for the liver being the seat of formation of immune body.

ALEXINS.

Fodor in (1886) (*65*) first determined the bactericidal action of normal rabbits' serum on anthrax bacilli. Flügge (1888) (*64*), recognising the power of sera to destroy bacteria in vitro, thought that this was an explanation of resistance to disease - the alexic or humoral theory of immunity. Baumgarten (1888) (*10*) thought that the destruction of bacteria by blood sera was simply/

simply due to the rapid change of medium, while Lubarsch (1889) (121) denied that this alexic power of the serum had anything to do with immunity, for he found that the serum of certain animals might be very bactericidal to a certain organism of disease, and yet the animal itself might be very susceptible to the disease and vice versa. Bastin (1892) (8) found, after the intravenous injection of a bacterial emulsion, that the decrease in the bactericidal power observed went parallel with a decrease in the number of leucocytes. V.Szekely and Szana (1892) (180) held that the destruction of the organisms in the blood is not due to anything like alexine, but that it is due to the rapid change in the medium of growth. Werigo (1892) ( ) showed that the injection, into the blood stream, of microbic cultures caused an oscillation in the bactericidal power of the serum, which went parallel with identical variations in the number of white cells in the blood. Denys and Kaisin (1893) (41) held that the destruction of the organisms by blood sera is not due to the rapid change of media, for they found that B.Coli transported from blood to blood undergoes as much diminution as when taken from gelatine to blood. Buchner (1894) (22), by his aleurone pleural exudates of leucocytes, which he washed, froze, and thawed, and then allowed to macerate, found that such exudate extracts were more bactericidal/

bactericidal against typhoid and coli than the serum and blood of the animal from which they were obtained. Havet (1894) (83) found that the partial or total disappearance, in dogs, of the white corpuscles, which succeeds an injection of microbial products into the blood, causes the partial or total disappearance of the bactericidal power, and the return of the bactericidal power coincides with a return of the leucocytes to the blood. Hahn (1895) (81) found Histon plasma as bactericidal as blood serum and concluded that the bactericidal substances are secreted by the leucocytes and circulate in the blood. He found further, that washed pleural exudates, treated by Buchner's method, had more bactericidal action, on staphylococci and typhoid bacilli, than the serum of the same animal. He added the isolated leucocytes to blood serum, and, by this treatment, found an increased bactericidal action, and concluded that the alexin is a vital secretion product of the leucocytes. Buchner (1897) (23) maintained his position that the leucocytes secrete the alexin or bactericidal stuff in vivo. Sawtchenko (1897) (165) experimented on the bactericidal power of rat serum ranthrax, as compared with the bactericidal power of leech plasma of the rat against the same organism, and thought that the alexin was not formed by the destruction/

destruction of the leucocytes on clotting, as he found the bactericidal power of the leech plasma and the serum to be the same. Daubler (1898) (39) found that the bactericidal substances of the leucocytes and the serum were certainly not identical. Nakanishi (1889) (136) found that defibrinated ox blood contains living leucocytes, with amoeboid movement, after a 10 days existence in vitro. (This rather tells against Metchnikoff'f view of phagolysis)* Von Dungern (1899) (48) found no haemolytic alexin in white corpuscle exudates. Schattenfroh (1899) (168) used leucocyte extracts from the pleura prepared after Buchner's method or by heating for ½ hr. at 55°56°C. He found that guinea pigs' leucocytes had no alexin against cholera vibrios, whilst the serum of the same animals was very active. The leucocyte extracts of some other animals were, however, active. Some of these leucocyte extracts lost their activity only by heating at 80-85°C for ½ hour, while the corresponding serum lost its activity by being kept at 55-60°C for ½ hour. Again these leucocyte extracts had no haemolytic power. In spite of these marked differences he however, held fast to the identity of the bactericidal substances of the leucocytes and the alexin of blood serum and thought in opposition to Buchner that the appearance of the alexin was due to the death of the leucocytes.

Laschtschenko/

*Metchnikoff holds that microcytase & macrocytase do not exist as such in the circulating plasma, but that they only appear extra cellularly and in the serum by injury to the microphages and macrophages. This injury he calls "Phagolysis".

Laschtschenko (1900) (106) showed that, if one treated rabbit's leucocytes with the heated serum of another species of animal, a bactericidal substance with all the characteristic properties of alexin was produced, and concluded that the foreign serum acted as a stimulant to the leucocytes, causing them to give off the alexin, and that this process did not take place in the leucocytes post mortem but intravitam. Bail (1900) (7) showed that the addition of leucocytes to the bactericidal serum from an animal of another species increased that bactericidal power, and he thought that, in leucocytes, there was present besides the heat resistant, also a heat labile, bactericidal substance. Löwit (1900) (119), by pounding leucocyte holding organs with glass powder, got a bactericidal substance. It was very heat resistant, and so not identical with the alexin of the serum. Trommsdorf (1901) (186) supported Buchner's position by an extension of Laschtschenko's experiment. He tested, as Laschtschenko had not done, the vitality of the leucocytes by means of their amoeboid movements & by the methylene blue reaction*. He found that it was impossible, under every condition, to extract a bactericidal alexin, by means of foreign sera, from rabbits' leucocytes. He had a large number of negative results, but the few positive ones were striking enough

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* A test for the vitality of a tissue - living tissues decolourising the methylene blue.

to warrant him in confirming Laschtschenko's statement and he concludes accordingly that the living leucocytes are the producers of alexin. Gengou (1901) (72.) tested the bactericidal action of rat serum and rat plasma, (obtained by drawing blood into paraffined tubes and deprived, as soon as possible, of leucocytes) and found that the plasma, so prepared, was much less bactericidal than the serum obtained by ordinary coagulation. He concluded that the white blood corpuscles were the seat of formation of alexin, but that they give it up only on "Phagolysis". Gengou (1901) (73.) further tested for the seat of origin of alexin by means of leucocyte extracts. By drawing off a pleural exudate, produced by injection of gluten-casein, 24 hours after this injection he obtained mostly polymorpho-nuclear leucocytes, while if he drew it in 2 to 3 days, he obtained mononuclears. He compared the bactericidal action of extracts of such exudates with that of the serum of the same animals against cholera, typhoid, coli, and anthrax and found that the polymorphs contained a larger amount of alexin than the serum, while the mononuclears contained none. Gruber (1901) (78.) found that specific haemolytic sera, when inactivated, cannot be activated again by leucocyte extracts, and he thus concluded that the/

the leucocytes were not the seat of origin of the complement. Schbayama (1901) (169) used the method of extracts, and found that extracts of the spleen, and the lymphatic glands, but not of the bone marrow, in normal guinea pigs, were haemolytic for dog's red blood corpuscles. Klein (1901) (96.) prepared organ extracts by rubbing up with sand in a mortar and then filtering. He tested the lytic action of these extracts on the red blood corpuscles of dog and rabbit. He found that the pancreas extract was constantly haemolytic,but that it could act also as an anti-haemolysin (1) against the haemolytic action of hen serum on rabbits corpuscles. None of the other organ extracts except the pancreas were haemolytic. Conradi (1901) (37.) got bactericidal and haemolytic substances, by allowing organs to undergo sterile autolysis and by pressing them thereafter with a Buchner's press. He got such substances in the juice of the liver, spleen, muscle, lungs and kidneys. The haemolytic substances haemolysed the animals own corpuscles, as well as foreign corpuscles, and further the haemolytic substance was heat resistant. This paper is important as it shows experimentally the method of origin of some of these active haemolytic and bactericidal bodies obtained by the method of organ extracts. Metchnikoff(1905) (129)thinks that there exist, in the same species of animal, two different/

different cytases.* One of these, the macrocytase obtained from the macrophages is found in the lymphoid organs and in the serum of the blood and acts more particularly on animal cells, but not at all on bacteria. Owing to this substance, extracts of the spleen, omentum, or lymphatic glands, which organs contain chiefly macrophages, dissolve red blood corpuscles more or less readily, but they are incapable of affecting bacteria. The microcytase is obtained from the microphages and it destroys easily bacteria, but has little or no effect on animal cells. Extracts of the bone marrow consist largely of microcytase. The microcytase will not lyse even red blood corpuscles to which specific fixative has been added. Neither of these two cytases are free in the serum as long as the leucocytes are intact. They only appear in the serum after phagolysis has taken place through injury to the leucocytes. In acquired immunity there is another substance, the fixative; but while the cytases injure the cells (bacterial or tissue) directly, the fixatives do not of themselves hurt it at all. By fixing themselves on to the cell they render it more susceptible to the action of the cytases. The fixatives are specific while the microcytases can attack all kinds of bacteria, and the macrocytases all kinds of animal cells. The fixatives are soluble ferments which are humoral i.e. they are free in the circulating plasma. The phagocytes are the producers of

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* He believes them to be of the nature of ferment hence the termination - ase.

the fixatives. Metchnikoff uses his term cytases in a very loose way, sometimes as equivalent to Buchner's alexins of organ extracts, (which they undoubtedly are) sometimes as equal to complement (to which they have not the slightest resemblance). Petterson (1902) (145) found that after the shedding of an animal's blood, the bactericidal power of the serum, in vitro, soon could increase and soon could decrease - increasing, because alexin comes from the leucocytes, decreasing, because the fibrin absorbs it. In certain animals, he found the giving off of alexin from the leucocytes, outside the body, to be so small, that the serum is inferior, in bactericidal action, to the plasma. In other animals, however, there is a large amount given off from the leucocytes. He used typhoid, coli and pyocyaneus in his experiment Tarassevitch (1902) (182) found a haemolysin in the macrophagic organs only - omentum, mesenteric glands and spleen. In addition, however, he found that extracts of the digestive glands, too, possess a haemolytic action. All the other organs, including the bone marrow, - the principal seat of formation of the microphages, are destitute of haemolytic power. As regards the bactericidal power, it is exactly the inverse - the bone marrow being the principal source of/

of it. The extracts of the microphages are not haemolytic, and even the addition of a specific haemo fixative to such an extract will not cause haemolysis. Extracts of macrophagic organs, containing much debris, when heated to 55°for 1 hour, in a certain number of cases, lose their solvent power for red blood corpuscles. Sometimes this temperature brings about merely a weakening of the cytatic action. To destroy it surely, the extract, must be heated, at 58-62°C. for an hour. If however, instead of heating the entire suspension, it is passed through filter paper before heating, the clear filtrate is deprived of its diastatic action even after it has only been heated at 55° for ¾ hour. The actions of the microphages and macrophages are to be attributed to two different cytases, the micro cytase acting against bacteria, and the macro cytase acting against animal cells. These two cytases pass into the humors, only after the destruction of the corresponding leucocytes. Up to this point Tarassevitch has been dealing with normal untreated animals. He then states that in contradistinction to the cytases the fixatives are found free in the plasmas, are secreted by the leucocytes and macrophagic organs, and that their function is to activate phagocytosis in vivo and in vitro.

Landsteiner (~~1902~~ 1898) (103) found that a specific haemolytic/

haemolytic serum, when inactivated, cannot be reactivated by exudate fluid or leucocyte extract; and argues from this, with some confusion of terms, that the alexin of the serum (which he takes as equivalent to complement) is not the same as the extract of leucocyte bactericidal stuff. Donath and Landsteiner (1903)(143) took guinea pigs organs, treated them after the emulsion method of Schibayama and Tarassevitch and added to the extracts, goose corpuscles and guinea pigs' corpuscles. They found that the extracts haemolysed even the guinea pigs' corpuscles. Lambotte, (1903) (101) by means of centrifuging blood in an isolated vein, to prevent coagulation, showed the presence of bactericidal alexin in the plasma. (This argues against the formation of alexin, from the leucocytes, by phagolysis, on clotting). Petrie (1903) (148) did not observe the destruction of B.Coli, typhoid, or Gaertner, by leucocytic estracts (obtained by freezing leucocytes, got by aleurone injection, with liquid air) while the serum of the animal, supplying the leucocytes, was quite active. Wolff (1903)(198) carefully sought for Metchnikoff's "phagolysis" working with pigeon corpuscles injected into the peritoneal cavity* and could never find any evidence of it. Pfeiffer also(1903) (152), along with several of his pupils, tried/

* practically a repetition of Metchnikoff's original and fundamental experiment, on which he based his theory of Phagolysis.

tried to observe this "phagolysis" by repetition of Metchnikoff's experiment. They however failed to observe it.

Walker (1903) (*189.*) found the alexin increasing in the serum according to the time it had stood in contact with the clot. On the other hand, he found that serum, separated from its clot, or defibrinated blood, centrifuged immediately, underwent a steady diminution, from the beginning, in its complement content. He considered that complement was a leucocytic product, only appearing in the blood as the result of a disintegration of leucocytes (see Henderson Smith, Schneider Infra).

Falloise and Dubois (1904) (*60*) produced a general leucocytosis and showed that there was no relation between the richness of the blood in leucocytes and the haemolytic and bactericidal power of the serum coming from this blood. Petterson (1906) (*147*), from experiments in the peritoneal cavity of guinea pigs, with typhoid, came to the conclusion that the guinea pigs leucocytes contain no active bactericidal substance against typhoid. Lambotte and Stiennoin (1906) (*102*) Metchnikoff had criticised Lambotte's experiments with the isolated vein, by saying that the mere shaking in the centrifuge was sufficient to cause a disintegration of the leucocytes and a setting free of alexin/

alexin. Lambotte and Stienoin , in this paper, showed that centrifuging had no effect on the vitality of the leucocytes because there was no diminution of their phagocytic activity to anthrax. They also tried the effect of variations of temperature on the vitality of the leucocytes. They criticised the work of Tarassevitch mentioned above, by saying that the extracts of digestive glands and of organs rich in macrophages did not always contain the haemolytic alexin; and further, as Taressevitch himself pointed out, the extracts of organs heated ½ hour at 55°- 56°C. preserved perfectly all their haemolytic activity. Again, if these extracts were made to act on sensitised* Red Blood corpuscles, they had practically no action. Extracts of polynuclear leucocytes were absolutely non haemolytic, whilst they could stop development of microbes. This they maintained in no way proves the existence of such a thing as a microcytase in the leucocytes for Tarassevitch has given no statement as to what happens when the leucocyte extracts are heated,& the same defect exists in Gengou's article on the origin of the alexins in the serum (see above). Lambotte and Stienoin tried to see if the leucocytes were the producers of alexin when suspended in normal/

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*With specific Immune body, which had been inactivated by heating.

normal saline or in heated serum and they never found any such production. They now repeated Tarassevitch and Gengou's experiments with reference to organ extracts not only with regard to the bactericidal action, but also with regard to the power of reactivating immune sera, rendered inactive by heating to 55-56°C for ½ hour. They found that the extracts of leucocytes had no bactericidal action in 8 out of 10 cases and found that they had no power of reactivating inactivated sera. The bactericidal actions of organ extracts they conclude, are not due to true alexins & they quote Pirenne (Central, für Bkt Bd XXXVI, 1904) as showing that the marked bactericidal action of the serum of the rat persists after the disappearance of the true alexin. Korshun (1907) (97) found that rabbit leucocyte extract was very bactericidal against typhoid bacilli, but that guinea pigs' leucocyte extract had only a very slight action. This bactericidal action is wholly removed by heating for ½ hour at 80-85°C. Complement, for typhoid ambo ceptor, is not present in this extract, and the bactericidal substances of the extract are not identical with the alexin. R.Schneider (1908) (173) found that the polymorpho nuclear leucocytes give off a bactericidal stuff - leucine - in vivo and in vitro/

vitro. This "leucine" however, is not identical with alexine. The haemolytic action of lymphatic gland extracts is therefore to be attributed to another substance than the haemolytic agent of the blood. Lazar (quoted by Schneider) acted on rabbits leucocytes with foreign sera and was able to obtain a staphyloccus destroying substance. Schneider obtained his leucocytes by injections of bouillon or aleurone, and prepared the extracts by Buchner's method. R.Schneider (1908) (/7/) tested Walker's statement (see above), that the serum,obtained by rapid defibrination and centrifuging, was weaker than that obtained by ordinary clotting. He tested the bactericidal power of such sera against typhoid bacilli and found exactly the opposite to what Walker had stated to be the case.He also showed that the temperature of separation of the serum had little effect on its alexin content. He kept rabbit blood at 38°C and 0°C.and hen blood at 41°C. and 0°C.and found the bactericidal action to be the same at both temperatures,so that at a temperature favourable for leucocytic life there was no increase and at a temperature unfavourable for the same, there was no decrease of the alexic power. On the contrary the alexic action of the serum is greater when the serum is soon removed from the clot and this result is possible due to prevention of that absorption of/

of the alexin by the clot which takes place on standing. Again he found that citrated plasma had as great an alexic power as the corresponding serum. He found the same to be the case with leech plasma as compared with its serum, and similarly with many other plasmata prepared with anticoagulants. With sodium fluoride plasma, he found that its bactericidal action was always stronger than that of the serum. He found no alexin present in the normal aqueous humour; but that a little time after puncture of the anterior chamber, it appeared, but that it rapidly disappeared again. Its appearance a short time after puncture was due to a transudation from the blood plasma, in which it exists free, while its absence normally and its rapid disappearance from the punctured anterior chamber, is due to its being a specific secretion. He consequently argues that the alexin is present in normal circulating plasma, and is not formed by phagolysis during clotting.

Many of the papers, afterwards given, which deal with the presence or absence of free complement in the circulating plasma, have a bearing on this question, as the <u>crux</u>, for the decision of the question, is whether or no the complement or alexin is given off into the serum during the clotting of the blood, and the extrusion of the serum. One such/

such paper is that of Löwit and Schwarz (vide Infra)

PROTECTIVE SUBSTANCES.

As regards the protective substances, formed in treated animals, and their source of origin, Landsteiner (1897) (*104*) found that the exudate fluid from a guinea pig immunised against mouse septicaemia, freed from cells, protected, in very small quantities, when injected previous to inoculation, a fresh mouse against infection with mouse septicaemia, but that, if the exudate, containing the leucocytes, were injected along with the bacilli there was not the slightest protective action. Blumreich and Jakoby (1895) (*14*) found that splenectomy produced a marked protection against pyocyaneus and diphtheria infections, but that it exerted no recognisable influence on pyocyaneus and diphtheria intoxisation, and that the blood after splenectomy gained a specific bactericidal action, but was not influenced as regards production of anti-toxin. The facts on which these conclusions are based are far from convincing. Wassermann and Takaki (1898) (*193*) by using extracts, found that the spleen, bone marrow, lymphatic glands and thymus are the seat of formation of protective substances. Wassermann (1899) (*192*) worked/

worked with pneumoccus and showed that the thymus, the spleen, and the bone marrow were the seat of formation of protective substances. Pfeiffer and Marx (1898) (153) used the method of extracts, and looked for the presence of protective substances in emulsions of certain organs of injected animals to a greater degree than in their blood serum. They worked with cholera vibrios which they injected subcutaneously. They got leucocytes, from these animals, by bleeding into oxalate solution and then by differential centrifugation of this oxalated blood. They increased the number of leucocytes in the blood by injection of spermine and killed the animal at the height of leucocytosis. Again they obtained leucocytes from pleural exudates and treated them by Buchner's method. These experiments gave no indication that the leucocytes of the blood were the producers or even only the carriers of the cholera protective substance. They next took rabbits immunised against cholera, bled them to death, emulsified the organs and tested the protective power of the various organs as compared with the serum. They found the spleen extract four times more active than the serum and eight times more active than the blood. While the cholera protective body is being formed, there is more/

more of it in the spleen and bone marrow, than in the lymphatic glands and lastly in the lung than in the serum. They found that cutting out of the spleen had no effect on the formation of cholera protective body. They attributed this to a vicarious function of the bone marrow and lymphatic glands. Deutsch (1899) (42.) used the method of emulsions, grinding the organs in a mortar sometimes with, sometimes without sand. He used typhoid. He found little anti-typhoid power in the liver, the kidney, the suprarenals or omentum. The value of the anti-typhoid power of the peritoneal exudate approaches sometimes that of the serum, but never surpasses it. In a quarter to one fifth of the cases, the bone marrow and in half of the cases the spleen is more active than the serum; so that the lymphoid organs are seen to take part in the formation of the protective substances in a minority of cases. He suggests therefore, that the bodies are formed in the blood itself. In splenectomised guinea pigs vaccinated against typhoid, the bone marrow extract is more efficacious than the serum. Splenectomy of the guinea pig, two or three days after injection, is followed by a diminution of the anti-typhoid power. Sometimes this is not so, and the injections of the spleen/

spleen of these last into other guinea pigs gives rise to specific agglutinins for typhoid. This shews that the bacilli have been fixed in the spleen, and he comes to the conclusion, on very unsatisfactory grounds it would seem, that the leucocytes are the seat of formation of the protective body. Tullio Muzzei (1907) (123) thinks that the spleen is of great importance with regard to infection with anthrax which is a septicaemia but not of so much importance with regard to tetanus and diphtheria which are more toxaemias.

AGGLUTININS.

In injected animals, the seat of origin of the agglutinins was investigated by Achard and Bensaude (1896) ( ). They used leeched blood from typhoid patients and found the clear plasma above the settled corpuscles quite agglutinative. Again they filtered off the leucocytes by means of special filter paper from the blood of an immunised donkey and found that the filtered blood was quite as agglutinative as the unfiltered. Again they washed the filtered off leucocytes with normal serum till no/

no more agglutinin was brought off in the washings then allowed the leucocytes to stand in the normal serum for some time and then tested this serum for agglutinin and found that it contained none. Widal and Sicard (1896) (196) added oxalate of Potassium to blood and found that the pure plasma had as great an agglutinative effect as the plasma leucocytes. They received the blood into oxalate of potassium solution in a sterilised collodion capsule, allowed it to settle and then ligatured above and below the layer of leucocytes and tested each layer for agglutinin and found that all the layers had the same agglutinating effect. Gourmont (1897) (38) tested the blood and organs of typhoid cadavers for agglutinin and almost invariably found the blood to contain most agglutinin. Rath (1898) (157) having injected killed typhoid cultures into animals, removed the spleen some time after. From these experiments and from testing emulsions of other organs, made by rubbing up with Kieselguhr, such as the spleen, bone marrow, and lymph glands, he concluded that the spleen, the lymph glands and the bone marrow, in rabbits, do not have a demonstrable influence on agglutination formation in artificial immunisation/

immunisation against typhoid. Arloing(1898) (3) infected animals with pneumo bacillus bovis and found the agglutination strongest in the blood serum and then in a diminishing degree in the lymph gland juice,bile,and liver juice. Van Emden (1898) (55) worked with B.aerogenes. He immunised animals with it and then tested,by the method of emulsions,for the agglutinating power of the various tissues. He concluded that if the lymphoid tissues were the chief seat of formation of the agglutinating substance, yet also other organs such as the liver, kidneys and lung, it may be in a much less degree, were also concerned. He found that,2 days after injection, the spleen juice had a much greater agglutinating power than the serum, but that after 3 days the reverse was the case. Foder and Rigler (1898) (66) found that agglutinins first made their appearance in the serum of guinea pigs rendered immune to typhoid bacillus, later it appeared in much less amount in the bile,extract of spleen,and liver. Deutsch (1899) (42) used the method of emulsions. He found in immunised animals,that the liver,kidneys,and suprarenals only contain traces of agglutinin. The lymphoid organs, as the spleen,bone marrow,and glands contain variable quantities without attaining to the value/

value of the serum. Splenectomy before injection did not hinder formation of agglutinin, but splenectomy, 3-5 days after injection, hindered slightly the formation of agglutinin. The lungs were the only organs which in immune guinea pigs had a higher agglutinating power than the serum, and further in the normal lung of the guinea pig, as well as of other animals, there were present normally agglutinins for typhoid. Gengou (1899) (7/) studied the agglutination of attenuated anthrax bacilli by the organs and fluids of non immunised guinea pigs. He found the largest quantity of agglutinin present in the blood and observes that the agglutinins here noted do not appear to come from the leucocytes or other cells of the body. He found, in dogs injected with anthrax, no agreement between the amount of agglutinin in the blood and the proportion of polymorphs.. Further, he filtered off the leucocytes and found that this filtration had no effect on the agglutinating power. Again he tested leucocyte extracts from animals, treated with anthrax vaccine No. 1, and found that the living leucocytes are neither the producers nor the holders of agglutinin. He tested the organs of/

of normal and immune animals by the emulsion method and found that it was very difficult to attribute to the organs or cells of the organism any intervention in the formation of agglutinin. He found that the organs had an agglutinative power much inferior to that of blood and which could be perfectly explained by the blood they contained. Jatta (1900) (94) worked with typhoid and used the method of organ emulsions. He found that 2 to 3 days after injection of typhoid, the agglutinating power in the spleen was much stronger than in the serum, but that later the reverse was the case. Ruffer and Crendiropoulo (1902) (162) found that extracts of leucocytes of normal and immune animals were more agglutinating than the sera of the same. They obtained their leucocytes by injections of gluten casein, and then treated them by Buchner's process. Sick (1903) (175) found that normally, agglutinin is, especially, found in the spleen liver and lungs. Immune agglutinin is found in all protoplasm equally with the blood plasma. He used the method of organ extracts. Levaditi (1903) (111) worked with the spirillum of hen septicaemia. He made emulsions of various organs of rabbits, which had been injected with infected hen blood, by triturating with/

with powdered glass &c. The fluid obtained finally contained cellular debris.* He came to the conclusion that the formation of antibodies against the spirilla in refractory organisms takes place in the leucopoietic organs in particular in the spleen, the bone marrow and the lymphatic glands: also in the omentum, which becomes a depot of leucocytes, when the injection is made intraperitoneally. The leucocytes, according to Levaditi, ought therefore to be considered as the principal if not the only source of the agglutinin. Figari (1904) (62) worked, in cows and horses, with the antitoxins and agglutinins for tubercle. He found that antitoxins and agglutinins are present in small quantities in the plasma, but that they are present in large amounts in the formed elements of the blood. He found that serum, obtained by coagulation, and the extract of formed elements were much more antitoxic and agglutinative than the serum obtained by whipping and immediate centrifuging. He argues from this that the leucocytes either prepare or store the agglutinin and antitoxin of tubercle.

Lowenstein (1905) (117) found that tubercle bacilli & killed typhoid bacilli, when injected subcutaneously or intravenously in the same amount, produce a large amount/

* possibility of the complement being absorbed and thus preventing any killing of the spirilla by a complement amboceptor complex.

amount of agglutinin, but that when injected into the anterior chamber of the eye they do not do so, and from this argues that a purely local infection can give rise to no agglutinin. Kraus and Schiffman (1906) (160) injected rabbits with killed typhoid, bled them to death after a time, and tested the agglutinating power of the serum as compared with organ extracts. They found that the agglutinin appeared much later in the organ extracts than in the serum. They conclude that contrary to what takes place in the bactericidal antibodies, which arise in the spleen, the bone marrow, and the lymphatic glands, the genesis of precipitin and agglutinin takes place in the vascular system. Tullio Mazzei (1907) (124) found that splenectomised guinea pigs do not develop much agglutinin against typhoid bacilli. Dreyer and Walker (1909) (47) found plasma to be more agglutinating than serum, owing to the absorption of the agglutinin by the clot. Consequently if serum is obtained equally agglutinating or more agglutinating than plasma, it follows that the increase in the agglutinative power is due to some factor partially or completely absent from the plasma. In their experiments they found that serum was more agglutinating than plasma during the period of latency/

latency and rise in the immunity curve of agglutination production; and this according to them corresponds to the time of maximum leucocytosis and they suggest that the difference between the plasma and the serum is due to the breaking up during clotting of this excess of leucocytes. They found further that by the injection of a totally different organism into an immunised animal the serum becomes stronger in agglutinin than the plasma. They explain this again by a leucocytosis and consider such facts as evidence for the leucocytes and leucocytic tissues, bone marrow, endothelium &c., being a source of agglutinin. Again they have found that plasma as contrasted with serum is relatively destitute of complement, and consider this as a support for their view that complement is derived from the leucocytes. Again in a rabbit immunised against B.Coli and whose immunity is past the maximum, if a dose of some other organism is given there is a new rise in the coli agglutinin. But,if the interval which has elapsed before the inoculation is made is of such a length that the immunity of the animal has ceased to be measurably greater than it was before immunisation was originally begun,no perceptible increase in agglutinin occurs. That is to say that/

that the cells and tissues which are thrown into activity by the new inoculation are precisely those which were already occupied in the formation of agglutinin, and when stimulated, therefore, lead to increased production. Now the tissues which are known always to be stimulated by bacterial inoculation are the leucocytic tissues of the body, bone marrow, lymphatic glands, endothelium &c. These observations tend to show that these tissues are a seat of formation of agglutinins.

I
PRECIPITINS.

Biondi (1902) (13) tried to find out the seat of origin of the precepitins in immunised rabbits. He bled the animals to death and washed out the organs with salt solution. He then emulsified them and found that they all contained precipitin, although the retroperitoneal glands appeared to contain more than the rest. Von Dungern (1903) (50) introduced albuminous substances into the anterior chamber of the eye, and concluded from his results that precipitin could be formed locally. He thought however, that the formed elements of the blood were the/

the chief seat of their formation. He injected into rabbits blood from other rabbits which had been injected with a definite albuminous substance and did not get a precepitin formed, from which he concludes that the former elements of the blood are the seat of formation.* Kraus and Levaditi (1904) (98) found, on intraperitoneal injection of horse serum that precepitin appeared in the great omentum before it appeared in the blood stream. This was due to it being formed by the leucocytes which had accumulated there. He tested by the method of organ extracts and he found that certain tissues from fresh animals, already precipitated the albumens used. He concluded that the white corpuscles were the seat of origin of the precepitins. Kraus and Schiffman (1905-6) (99) found as Kraus and Levaditi had done that the omentum gave, after an intraperitoneal injection of horse serum, a precepitin before any could be got in the blood serum or in the other organs. They investigated the organs as a possible source of precepitin by means of saline extracts. They used rabbits and injected them subcutaneously intra-venously and intraperitoneally with horse serum/

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*because according to him the formed element of the blood had taken up the albumenous and so altered it that when this blood was injected into another animal it gave rise to no precipitin.

serum. Their organ extracts did not show any precepitating power and they came to the conclusion that the source of the precepitin is in the vascular system probably in the endothelia. They removed the spleen from animals and found that the splenectomised animals produced precepitin quite as well as the controls. Brezina (1905) (19) injected guinea pigs with splenolytic and myelolytic sera and found that they had lost the power of antibody formation and from this concludes that the spleen and bone marrow were the seat of origin of the antibody. Cantacuzene (1907) (31) investigated the seat of origin of precepitins by using the method of organ extracts. He found in normal rabbits,already present,small quantities of precepitins for foreign sera and to increase the amount it was necessary to excite the activity of the organs which were their seat of origin. An injection of aleurone could do this. These precepitins were non specific and their characteristic is that their increase occurs in a few hours after the aleurone injection. Their seats of formation are the spleen, the bone marrow, the mesenteric glands and the cells forming them are the leucocytes probably the mononuclears. When however, an antigen, such as horse serum,is injected there is produced a precepitin/

precepitin which is specific and which takes much longer than the normal non specific precepitins to develop. These specific precepitins have the same origin as the non specific, and are formed from the same cells, the mononuclears. His technique was emulsification insaline, maceration, centrifugation and filtering through filter paper. He obtained evidence of the appearance of precepitin in the spleen, mesenteric glands, and bone marrow several days before its appearance in the blood. He explains the different results obtained by Kraus and Schiffmann by stating that his own extracts were stronger than theirs. Further, Cantacuzene injected rabbits with horse serum and afterwards injected them intraperitoneally with aleurone. He made extracts of the fibrinous deposit on the omentum, and found that precepitin appeared on it before it appeared in the blood. The fibrinous deposit consists of polymorphs and mononuclears and he argues from this for a local production of precipitin. He further finds that, in a subcutaneous aleurone abscess in a rabbit, which had been injected with horse serum, the pus contains precepitin 3 or 4 days before it appears in the blood, and he concludes from this that the leucocytes are the elements which form the precepitins.

Haemolysins/

ARTIFICIAL HAEMOLYSINS. AND BACTERIOLYSINS.*

Finally as regards the seat or origin of artificial haemolysins and bacteriolysins. Havet (1894) (84) found that in active infection with living cultures, the stage of hypo leucocytosis is accompanied by a diminution of bactericidal power in the blood and hyperleucocytosis by an increase. Moxter (1899) (133) found, in leucocytic extracts from animals immunised against cholera, much less bactericidal substances than in the serum of the same animal. London (1901) (116.) splenectomised guinea-pigs and commenced treating them at varying times after the operation with foreign blood injections. In some of the splenectomised animals, after having removed the spleen he cut it up and put it back again into the peritoneal cavity. In none of the animals did he get the formation of any haemolysin, and from this concludes that the spleen plays a role of great importance in the production of haemolysins.

Gengou (1901) (74) compared the bactericidal power of exudations rich in microphages and of exudations rich in macrophages with that of the boood serum of the same animal. The bactericidal power of the extract of microphages was always greater than that/

*taken together because of their essential simularity in action.

that of the corresponding serum. The extract of macrophages shewed no bactericidal power. Bulloch (1901) (24) from counting the leucocytes in rabbits after injection of ox blood corpuscles, concludes that the immune body is formed from the lymphocytes. Tarassevitch (1902) (183) disagreed with London, and held that, in splenectomised animals, the haemolysin was formed to the same degree as in the control guinea pigs. He thought that the property of forming immune body did not belong to a single organ, but belonged to the whole macrophagic system. Cantacuzene (1902) (29.) attributes to the omentum the seat of formation of the antibody to liver cells. Paton and Goodall (1903) (143.) found that removal of the spleen was not followed by an rise in the number of erythrocytes or leucocytes. Excess of erythrocytes added to the blood by injection of the defibrinated blood of an animal of the same species disappeared at the same rate in an animal with and in an animal without its spleen. There is thus no evidence that the spleen has any active haemolytic function towards its own red blood corpuscles. Jakuschawitsch (1906) (93.) found that when splenectomised animals were injected with foreign red blood corpuscles , they produced immune body, as well as, and sometimes better than the controls, and it makes no difference on the strength/

strength of the haemolytic sera on which day after the splenectomy, the 3rd or the 30th, the inoculations were begun. He thinks that the greater potency of the serum of some splenectomised animals is due to the leucocytosis caused by the splenectomy which indicates an increased action of the bone marrow. He thinks, therefore, that the haemolysin is not entirely produced by the spleen. Tullio Mazzei (1907) (124) finds that the bacteriolytic power of the serum of splenectomised guinea pigs is the same as that of normal guinea pigs. Wassmüth (1907) (190) finds that rabbit leucocytes are able to suspend up to a certain point the globulicidal property of rabbit serum with regard to guinea pigs corpuscles.* This power is found in dead leucocytes as well as living ones. Szokalski (1908) (181) thinks that one cannot attribute to the spleen an exclusive role in the formation of haemolysins as the splenectomised animals produce haemolysins as well as, if not better than normal animals. If one injects the animal at the operation, the operation itself causes 3 or 4 days delay in the appearance of the haemolysin.

SUMMARY AND CRITICISM OF LITERATURE.

When one comes to make a critical survey of/

*by absorption of complement?

of the literature given above, one recognisses that the key note of the situation is confusion, - confusion as to the meaning of certain terms, or at any rate a confused use of these terms,confusion as to the legitimate deductions to be drawn from a given experiment, and,lastly,confusion as to the technique and experimental methods to be used to prove or disprove a certain assumption. First with regard to the confused use of terms. The word,alexine,was first used by Buchner to indicate a substance in the serum of normal animals, which had the power of destroying bacteria and which was destroyed by heating to 55°for an hour. Searching for the seat of origin of this substance in normal animals, by the method of extracting organs, he obtained substances in certain organ extracts - e.g. - leucocytes - which had the power of destroying bacteria. To these he applied the name of alexins. They resemble the alexin of normal sera in their bactericidal action and in some cases by their being destroyed by heating to 55°C. for ½ hour. We could suppose that, just as happens for many of the alexins of normal sera and for artificial immune sera, this destruction of action was due to a destruction of complement (the thermo labile substance of normal sera, which unites with the fixative of normal and immune sera to cause bacteriolysis and/

and haemolysis) and that the bactericidal action could be brought back to these organ extracts, as in the case of inactivated normal serum alexin, by the addition of complement of itself without action on the bacteria used. But in no case, recorded in the literature, as far as I can see, has this activation of the inactivated organ extracts been accomplished, — a fact which had it been accomplished would have gone far to show the essential similarity in mode of action of the bacteriolysins of the alexin of normal sera and the alexine of these organ extracts. Further, as noted above, a great many of these organ extracts resist for a long time without injury , a much higher temperature than that which is necessary for the inactivation of the serum alexin: Grubers "anthra cocidin"* is an example of such heat resistant substances occurring in the serum itself: and again Conradi (1901) (37) has shown that sterile autolysates of organs, and organ extracts obtained by means of Buchner's press; (the organs used being liver, spleen muscle, lungs, and kidneys) possessed bactericidal and haemolytic powers. The haemolytic action occurred with the animals own corpuscles as well as with foreign corpuscles, and further these extracts of Conradi's were very heat resistant. Metchnikoff's microcytatic/

* Gruber finds a heat resistant substance in the serum which destroys anthrax bacilli. This he calls "anthra cocidin" and he holds that it is derived from the blood platelets.

microcytatic and macrocytatic organ extracts are alexins in the sense of Buchner's extracts and the same criticism applies to them.

By the progress of research, it was shown that the alexins of normal sera and the sera of artificially immune animals consisted of two factors, the thermo labile factor or complement and the thermo stable factor, the amboceptor or fixative. These two substances acting conjointly effect the lysis of bacteria and red blood corpuscles, while acting singly neither of them have any effect on these cells. Even after this knowledge of the essentially twofold nature of the action of immune substances had been gained, experiments were done with these organ extracts* and deductions drawn, from results obtained with them, as to the seat of origin of the two substances in normal and immune sera which by their united action, produce lysis of bacteria and red blood cells. Metchnikoff, in particular, continues to use the terms microcytase, indicating by this the substance, of itself bactericidal, contained in extracts of polymorphs; and macrocytase, by this indicating the haemolytic substance contained in extracts of mononuclears. He further speaks about the fixative (amboceptor, Immune body of others) as sensitising the/

* which have never been shown to consist of two substances.

the corresponding cell for phagocytosis. Lysis in immune sera extracellularly, according to him can only take place by phagolysis and the shedding off of, according to him the macrocytase and microcytase, which he,in this connection,tacitly assumes to be the same as the complement of other authors. But the microcytase and macrocytase, according to Metchnikoff's own showing, just as the alexic extracts of Buchner, are in no way identical with the complement. Hence one would assume that Metchnikoff,in immune sera, finds not only microcytase macrocytase, and immune body but also the complement of other authors, while the majority of investigators find only complement and immune body. Hence the most utter confusion, and,as Metchnikoff's views have dominated the subject of immunity up to the present time, there is consequently great confusion in the use of terms and in the methods of research.

With regard to the second element of confusion - that as to the legitimate deductions to be drawn from a given experiment,there are few instances in the literature recorded,in which,from the writers own protocol,three or four different conclusions other than that which the author has drawn,including a diametrically opposite one,could not be drawn. And finally/

finally,owing to the utter confusion as to the issues at stake,experiments have been devised which if they were successful,as experiments,in the highest degree,would go to prove something far other than that which the author intended them to do. Very often again from an entire lack of appreciation of the limits of experiment and experimental error, experiments have been undertaken,which a consideration of some fundamental principles would have shown to be impossible - a notable example of this being the endeavour to find the source of origin of immune body by means of organ extracts. For,in the first place,there is here a great unwarranted assumption explicable no doubt from a false analogy with the pancreas and other glands, but these are definite glands with a definite glandular structure and a definite secretion, while the spleen,bone marrow etc., have none of these characteristics. Again there is the practical difficulty, that tissue extracts absorb complement in large quantities which action may entirely obscure a very large quantity of fixative in an organ, as will be seen from certain experiments of mine,done on the liver, before I fully appreciated this fact. Further in these organ extracts/

extracts one may get the signal, so to speak, of the action one is looking for but that signal may be due to an entirely different mechanism* - e.g. haemolysis in certain organ extracts, being due to a heat resistant body and again the occurrence of agglutinins and precipitins in the tissues of normal animals. Again these extracts are always turbid and for the detection of the fine difference in turbidity due to the first traces of precipitin appearing in an organ before it appears in the serum an extremely fine discrimination would be more than necessary. When the precipitin or other antibody have appeared in the serum comparison of serum and organ extract, and consequent incrimination of certain organs is impossible, because there is no standard of comparison and no data for judging how much extract is to be taken as equivalent to a certain amount of serum: and besides there is always the fallacy of the blood contained in the organs. The absolutely conflicting results given in the literature above by equally competent investigators is the only further comment I would make on this method of research. For these reasons I have thought it waste of time to attempt to refute or corroborate their results.

Literature/

* to the one which one is investigating.

LITERATURE OF FEEDING EXPERIMENTS.

As the subject of feeding experiments in relation to the production of immune body is an important one, and as I have several experiments of this nature to record I shall now give the literature referring to this subject. Ehrlich (1891) (53) produced immunity in animals against ricin and abrin injections subcutaneously by feeding them on these substances. Fraser (1895) (68) by feeding animals with snake venoms immunised them against several times the minimal lethal dose subcutaneously. Uhlenhuth (1900) (166) fed rabbits by means of a stomach tube on egg white. He got a precipitin in their blood 24 days after commencing this treatment. Metalnikoff (1901) (125) fed rats on rabbit and horse blood and produced haemolysins and agglutinins for rabbit and horse blood. He also fed rabbits on horse blood and produced too a specific haemolysin. Hamburger(1902) (82) found the rapid appearance of egg white in the serum of a dog fed therewith although no precipitins were formed in this animal. Ascoli (1903) (6) demonstrated egg white and substances from roast fowl in the lymph of dogs fed therewith. The/

The serum of human subjects fed on roast beef also contained substances which were precipitated by an homologous antiserum for ox meat. Where precipitins were contained in the serum of an animal thus fed, the amount of precipitins therein underwent considerable oscillation when a corresponding food was given. Tchitchkine (1904) (165) found that when he fed adult rabbits on typhoid bacilli they produced agglutinins, fixatives, and sometimes precipitins. When young rabbits were treated they did not produce any of them. Ganghofner (1904) (70) found that the giving of foreign albumen by the mouth to new born sucklings caused formation of an antibody the production of which is the cause of the animal getting thin and even of its death. Celler and Hamburger (1905) (35) criticise Metalnikoff's experiments by saying that normal rat serum is haemolytic to horses blood. They fed rats with ox blood for a month, and found no Immune body/ox blood in the rat serum even when the feeding was conducted in the natural way. The ingestion of foreign albumen or milk* does not give rise to the formation of an antibody. Moro (1906) (132) records the case of an atrophied infant of 4½ months, which had been fed/

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*according to them.

fed on cows milk&whose blood precipitated cows milk in the dilution 1 in 80. Arinkin (St.Petersburg) by feeding dogs by mouth with mutton fat got it stored up as such in the animal. Cantacuzene (1907) (32) gave rabbits,by means of a sound,horse serum by the stomach. In twenty one experiments he had sixteen positive results and five negative results of the appearance of a precipitin in the serum. He used adult animals and gave them 140cc in 5 doses the doses being given with the interval of a week. Ceaparu (1908) (34) quotes Römer, and Uffenheimer as having shown that the intestinal wall is permeable to a certain number of albumenoid antigens, and Ascoli, Bonfanti and Figano, Michaelis and Oppenheimer and Cantacuzene as having shown that the white of egg and normal horse serum given by the mouth of adult animals traverses the intestinal wall and gives rise in the blood to the formation of specific precipitins. Ceaparu found that specific haemolytic sera when given by the mouth in rabbits and dogs,were absorbed and caused haemolysis of the animals red blood corpuscles. (It is interesting in this connection to remember the modern method of giving antitoxin by the rectum in treatment/

treatment of diphtheria). Rosenau and Anderson (1908) (161) sensitised guinea pigs against an injection of horse serum by feeding them for several days on horse meat or dried horse serum. Steinberg (1908) (178) found in the case of a man fed with horse serum the production of precipitins. Rabbits fed with horse or ox serum did not show precipitins. When they were given typhoid cultures by the rectum they produced agglutinins. Claudio Ferni (1908) (61) immunised mice and rats against rabies by feeding them on rabies cords and on normal cords. Breton & Petit (1908) (16) vaccinated against diphtheria and produced antitoxin by giving diptheria cultures by the mouth. Calmette and Breton (1908) (26) found that tuberculine was absorbed quite well by the rectum and gives the tuberculine reaction in tuberculous patients. Breton and Petit(1908) (17) produced,by injecting into the rectum washed spores of tetanus and tetanus toxin in large amounts,a complement deviator and an antihaemolysine. Breton and Massol (1908) (18.) tested the absorption of cobra venom and its antivenine by the rectum, & found that the toxin was absorbed more rapidly by the large intestine than from a subcutaneous injection; but that the antivenin was hardly absorbed at all. Petit/

Petit and Jean Minet (1908) (144) obtained precipitins in the blood of rabbits by injecting into their recta, with a sound, egg albumen. Similarly he obtained in a man a precipitin by giving him per rectum, diluted egg white. The egg albumen was also obtained in the urine. Formario (1908) (67.) vaccinated rats and rabbits against plague by feeding them on cultures of plague. Rabbits were quite easily vaccinated. By rectal feeding the animals were easily vaccinated. In all the animals treated by the mouth or rectum, the phenomenon of deviation of complement was obtained and in all the animals the opsonic index was raised to plague.

SUMMARY AND CRITICISM OF FEEDING EXPERIMENTS.

From these it will be seen that there is a good deal of evidence that animals can be vaccinated by feeding against substances with which their organism could not have been familiar. And this very unfamiliarity might explain the absence in the sera of ordinary individuals of precipitins to the various albuminous substances of their food. The organism has become accustomed to them and no longer/

longer reacts by the methods it uses to more unfamiliar substances, and there is experimental evidence for such a view. Tchistovitch (1899) (184) found that long continued treatment of rabbits with eel serum leads to the disappearance of the precipitin for the eel serum from the blood of the rabbit. Nuttall (1901) (142) again found that the precipitin disappeared from the blood of rabbits treated too long with ox and sheep serum. Further Calmette and Breton (1908) (27) found that if they treated animals for a long time with foreign blood corpuscles the amount of haemolysin began to decrease, but if the animals were allowed to rest for some time, they produced a very active serum again after very few injections. Remy (1906) (159) finds that after injecting rabbits with hen corpuscles, the immune body reaches a maximum after three injections, and that further injections cause a fall. These last two are only particular instances of a fairly well established general rule. Again the fact that a precipitin cannot be produced in an animal by the injection of the serum of a kindred species, that in certain animal species as the dog precipitins are with difficulty produced to any foreign/

foreign albumen and that in other species such as the rabbit, there is an abnormal proclivity towards this formation are of importance in this connection.

IS THE SPLEEN, THYROID, KIDNEY, OR BONE MARROW AND LYMPHATIC APPARATUS THE SEAT OF FORMATION OF THE IMMUNE BODY?

To determine this, advantage was taken of the fact that a rabbit when injected intravenously with washed ox blood corpuscles produces Immune body in its serum first on or about the 3rd day, as Sachs (163) (1903) Bulloch (24) (1901) and I myself found previous to undertaking the experiments. This time standard had not been previously used in the investigation of this question. Rabbits were taken, their serum was ascertained very carefully to be free from normal lysin for ox blood corpuscles various organs were then removed, the animals again tested for normal lysin, they were then injected intravenously with 3cc washed O.B.C. and daily examined for the appearance of Immune body. In the case of the leucocyte forming organs, the animals selected as before were injected intravenously, and at short intervals their leucocytes were counted absolutely and differentially. These experiments involved much time, as numerous rabbits had to be examined before sufficient could be got without/

without normal lysin in their blood and this number was further reduced by deaths during the operation. As a matter of fact the test to which I subjected the animals, was that their serum in doses of 1cc should not show a trace of haemolysis with 1cc 1% suspension of ox blood corpuscles, frequently indicated in this research by the initial letters O.B.C. in 2 hours at 37°C: and that their serum should not do this on 3 occasions at intervals of 3 days with 3 different samples of blood.

EXPERIMENT I.

Rabbit, buck, selected as above, injected 2 weeks after removal of spleen with 3cc washed O.B.C. +2cc saline intravenously into ear vein. Blood tested daily (1cc serum + 1cc 1% O.B.C. 2 hours at 37°C) for Immune body, Immune body appeared first on the 3rd day.

EXPERIMENT II.

Rabbit, buck, selected as above, injected two weeks after removal of thyroid with 3cc washed O.B.C.+ 2cc saline intravenously into ear vein. Blood tested daily as before Immune body appeared first on/

on the 3rd day.

EXPERIMENT III.

Rabbit, buck, selected as above, injected two weeks after removal of spleen and thyroid with 3cc washed O.B.C.+ 2cc saline intravenously into ear vein. Blood tested daily as before, Immune body appeared first on the 3rd day.

EXPERIMENT IV.

Rabbit, buck, selected as before, injected two weeks after removal of one kidney with 3cc washed O.B.C.+ 2cc saline intravenously into ear vein. Blood tested daily as before, Immune body appeared first on the 3rd day.

EXPERIMENT V.

Rabbit, buck, selected as before, in addition leucocytes ascertained to vary between 9,000 and 12,000. Spleen removed and 2 days after its removal rabbit injected intravenously with 3cc washed O.B.C. 2cc saline. Immune body appeared first in 3 days, and there never was any leucocytosis. (This experiment was done after the experiments next given had been done).

The/

The next set of experiments deal with the question of a leucocytosis during the formation of Immune body.

LITERATURE.

Bulloch (1901) (24) investigated this question and came to the conclusion that the Immune body was formed from the mononuclears of the blood. Hunter (1908) (92) made observations on the behaviour of leucocytes during the formation of precipitins. He took great care to exclude sepsis, and his injection solutions were sterilised by heating to 56°C. on 8 successive days. He counted the leucocytes daily and found a general rise in the leucocyte count during the course of immunisation. There was a special rise after each injection, and the rise was as a rule greater and greater with each injection and each increase was of relatively short duration and was succeeded by a fall to nearly the previous level. The polymorphs were those which increased most and this was most marked with ox serum. His leucocyte counts varied between 7,000 and 30,000. He concludes by saying if the leucocytes are not the seat of origin of the precipitin it is impossible/

impossible at present to say what is. He has examined from this point of view, the spleen, liver, bone marrow and kidney. Extracts were made of these organs in the fresh as well as the dried condition, after they had been as thoroughly as possible freed from blood. None of these extracts had any precipitating power. Michaelis and Oppenheimer (1902) (130) found a polymorph leucocytosis on the injection of ox serum into the blood of rabbits. This leucocytosis was much greater in immune rabbits than in fresh ones. Fleischmann and Michaelis (1902) (63.) found after the injection of albumen into an animal, at first a leucopenia and later a hyper/leucocytosis. Holmes (1906) (91.) injected various animal cells and sera into rabbits and observed the behaviour of the leucocytes. He injected 10cc washed sheeps corpuscles, intraperitoneally into a rabbit. This rabbit was in no sense of the word a normal rabbit as it had just recovered from a leucocytosis caused by the injection of living typhoid. In spite of this its leucocytes did not rise above 10,000 which is within the limits for all rabbits and for the same rabbit at different times. Again he injected 10cc sheep serum intraperitoneally into a rabbit and got a/

a marked leucocytosis especially mononuclear. He concludes that the injection, in rabbits, of dead non-toxic animal matter such as animal cells and red blood corpuscles produces a reaction exclusively affecting the mononuclear leucocytes, and that the reaction to the injection of foreign serum is mainly mononuclear.

CONDITION OF NORMAL RABBITS' LEUCOCYTES.

As there seemed to be a good deal of doubt about the normal state of the leucocytes in normal animals, I thought it worth while before going on to investigate the changes produced in them by injections of ox blood, to determine what effect feeding, time of day &c., had on the number per Cmm. and on the differential count.

LITERATURE.

Brinkerhoff and Tyzzer (1902) (20) give the following figures for the rabbits leucocytes. Amphophiles 40-50%, Eosinophiles .5-1%; Mast cells 4-8%; Lymphocytes 45-55%, Large mononuclears 2-8%. They/

They maintain that the following factors must be considered in drawing inferences from variations in the leucocyte count - loss of body heat shock, fasting and feeding, pregnancy. In a large number of Brinkerhoff and Tizzer's normal rabbits the leucocyte counts varied between 4,600 and 13,400. Holmes agrees with this latter statement. Lisin (1908) (115) gives the following figures for leucocyte counts in the normal rabbit. The number of leucocytes per Cmm. may vary between 6,000 and 14,000 in the normal rabbit. For the differential count he gives the following figures. Mast cells 2-12% Eosinophiles 0·5-4%, Large mononuclears 2-5%, Lymphocytes and small mononuclears 37-60%, neutrophile polymorphs 32-45%.

WHAT EFFECT HAS VARIATION OF THE ORDINARY LABORATORY FEEDING, TIME OF DAY &C., ON THE ABSOLUTE AND DIFFERENTIAL COUNT OF RABBITS LEUCOCYTES?.

In determining this, the leucocytes were counted by means of Zappert modification of the Thoma Zeiss apparatus. Two different dilutions

(10/

10 large squares) were counted at each estimation and the number of leucocytes per Cmm. obtained by multiplying the average obtained for one large square by 200. The differential counts were made by taking a drop of blood on one slide, spreading it by means of the edge of another slide, drying, fixing and staining by Leishman. To obtain a good average of the whole slide, 400 cells were counted, 100 being taken from each of the two ends of the slide, and from each of the two sides. The leucocytes were counted simply as polymorphs and mononuclears.

EXPERIMENT/

EXPERIMENT I. Rabbit, buck. RABBIT 1. Mark Cage 232

HR.	WGT.	GAIN IN WT.	REC-TAL 1°	CAB-BAGE.	CORN & BRAN	LEUCO-CYTES.	POLYS.	MONOS.	REMARKS
9·20	2300		39	Since last		8200	40	60	(Fed on corn &
9·20A	2370	+70	39·2	observation		7400	26	74	bran & cabbage
1·40P	2460	+90	39·1			8800			at 10 o'clock.
11·P	2415	-45							
10A	2367	-48	.	.	.	.	.	.	(Before being fed
									(after this given
									at 10.30 360 gr.
2P	2455	+88					17	83	cab. & weighed
6·30P	2510	+55	39·7			7200	20	80	quantity corn &
10 P	2430	-80							bran.
9·30A	2385	-45	.	.	.	.	.	.	(Had eaten 290grs
2·30P	2435	+50	39·7	103	43	8200	31	69	cabbage & 97 grs
6P	2445	+10		62	7				corn & bran in
10P	2450	+5		75	53				24 hours.
9·40A	2435	-15	39·5	60	32	10000	17	83	
2P	2450	+15		53	30				
10P	2530	+80		122	70				
9A	2460	-70		33	20				
2P	2510	+50		93	17				
6P	2505	-5	40°	64	19	7600	28	72	
11P	2487	-18		76	12				
9A	2420	-67	39·5	55	22	7600	18	82	
2P	2470	+50	39·7	107	13	8800	23	77	
10P	2490	+20		135	50				
9A	2445	-45	39·9	58	33	9000	34	66	
2P	2470	+25	40	88	25	7000	21	79	
7P	2510	+40	39°	78	26	6200	28	72	
10P	2470	-40		31	14				
·45A	2457	-13	40°	103	38	9000	13	87	
2P	2495	+38	40°C	75	22	7000	18	82	
6P	2520	+25	39·9°	73	25	6800	24	76	
10P	2497	-23		38	23				
10A	2490	- 7		68	38				
10A	2510	+20	39·6°	220	108	9400	12	88	
2P	2530	+20	39·8	43	20	10600	23	77	(all food removed
6P	2505	-25	39·7			8000	17	83	from cages.
10P	2490	-15							
10A	2405	-85	39·2			6800	18	72	
2P	2500	+95	39·8	95	22	7600	23	77	(all food removed
									from cages.

RABBIT I.

EXPERIMENT I. Rabbit, buck. RABBIT I Mark Cage 23^2.

HR.	WGT.	GAIN IN WT.	RECTAL 1^0	CABBAGE	CORN & BRAN	LEUCOCYTES	POLYS.	MONOS.	REMARKS.
6P	2445	-55	39·6			7800	26	74	
10P	2420	-25							
10A	2390	-30	39·5			7400	14	86	
2P	2445	+55	39·5	92	25	8000	25	75	(all food removed.
6P	2420	-25	39·6			7600	21	79	
10P	2380	-40							
10A	2380	0	39·5			6800	17	83	
2P	2430	+50	39·5	98	23	7000	24	76	(all food re- (moved from (cage.
6P	2415	-15	39·6			6200	25	75	
10P	2400	-15							
10A	2350	-50	39·5			7400	22	78	
									(3 days inter- (val to rest (rabbits.
									(Given cabbage (corn & bran to (excess, food (in the cages all (the time.
10A	2300		39·6			10200	22	78	
2P						7800	17	83	
10A	2350		39·6			10200	17	83	
3P									(The rabbit was (now injected sub- (cutaneously with (O.B.C. The re- (sults will be (recorded later.

EXPERIMENT II. Rabbit, buck. RABBIT 2. Mark Cage 21^2

HR.	WGT.	GAIN IN WT.	RECTAL 1^0	CABBAGE	CORN & BRAN	LEUCOCYTES	POLYS.	MONOS.	REMARKS.
10A							28	72	(fed at 10 o'clo
									(on corn bran &
									(cabbage.
8P							23	77	
10A	1510		39·2			12400	24	76	
2P	1580	+70	39·2			14400	11	89	
11P	1550	−30							
10A	1515	−35							
12P	1595	+80							
6P	1620	+25	40^0C.			10600	16	84	
10P	1620	0							
9A	1560	−60		290	45				(had eaten 290
3P	1570	+10	39·9	68	38	12600	19	81	(grammes cabbage
7P	1580	+10		73	14				(& 45 grammes
10P	1575	−5		79	33				(corn & bran in
10A	1570	− 5	39·2	80	40	10600	14	86	(24 hrs.
2P	1570	0		70	0				
10P	1615	+45		190	40				
10A	1555	−60		40	15				
2P	1620	+65		78	17				
7P	1612	− 8	39·9	78	13	13800	15	85	
11P	1630	+18		95	18				
10A	1580	−50	39·2	49	14	14000	14	86	
2P	1635	+55	39·5	73	14	14600	16	84	
11P	1675	+40		152	36				
10A	1620	−55	39·2	75	15	13400			
2P	1645	+25	39·5	75	12	14800			
8P	1650	+ 5	39·9	95	15	13000			
11P	1607	−43		40	0				
10A	1597	−10	39·5	90	5	12800	21	79	
2P	1610	+13	39·5	70	3	13800	32	68	
7P	1605	− 5	39·8	78	12	15200	31	69	
10P	1620	+15		89	15				
10A	1580	−40		63	12				
10A	1617	+37	39·2	258	60	13400	20	80	
2P	1655	+38	39·7	63	15	14200	16	84	(all food removed
6P	1620	−35	39·5			12200	18	82	(from cage.
10P	1590	−30							

EXPERIMENT II. Rabbit, Buck. RABBIT 2 = 21^2

HR.	WGT.	GAIN in WT.	REC-TAL 1^0	CAB-BAGE	CORN & BRAN	LEUCO-CYTES	POLYS.	MONOS.	REMARKS.
)A	1565	-25	39·2			10000	14	86	
3P	1625	+60	39·2	75	18	13000	16	84	(all food removed from cage
5P	1575	-50	39·5			12400	15	85	
.P	1535	-40							
)A	1520	-15	39^0			10600	15	85	
3P	1620	+100	39·5	135	12	14200			(all food removed from cage
5P	1540	-80	39·2			11800			
.P	1530	-10							
)A	1530	0	39·2			12400	19	81	
3P	1600	+70	39·7	103	15	14400	11	89	(all food removed from cage
5P	1530	+70	39·5			12600	13	87	
.P	1498	-32							
)A	1485	-13	39·2			12600	10	90	
									(3 days interval
.	.	.	. .	.	.	. .	.	.	(Fed on corn, bran & cabbage to excess.
)A	1515		39·7			14600	11	89	
3P			40·2			13600	21	79	

(Killed and found to be quite healthy in all its organs.

EXPERIMENT III. Rabbit, buck. RABBIT 3. Mark cage 22^2

HR.	WGT.	GAIN IN WT.	RECTAL t°	CABBAGE	CORN & BRAN	LEUCOCYTES	POLYS.	MONOS.	REMARKS.
10A	2020		40°C			9200	33	67	(fed at 10
2P	2060		39·1			8800	42	58	(o'clock on corn
11P	2050	-10							(bran & cabbage.
10A	2030	-20							
2P	2067	+47							
7P	2050	-17	39·8			6400	47	53	
10P	2047	-3							
10A	2030	-13		290	20				(had eaten 290gr
2P	2030	0	39·9	68	13	12600	34	66	(cabbage & 20 gr
7P	2015	-15		56	3				(corn & bran
10P	2010	- 5		11	26				(in 24 hours.
10A	2020	+10	40·2	130	49	6400	40	60	
2P	2045	+25		65	0				
10P	2020	-25		128	20				
10A	2005	-15		107	35				
2P	2050	+45		108	3				
7P	2037	-13	39·9	77	7	8200	44	56	
11P	2032	-5		42	50				
10A	2015	-17	39·9	54	27	8200	34	66	
2P	2050	+35	39·7	76	2	8400	33	67	
10P	2050	0		109	25				
10A	2050	0	40·1	115	30	7600	30	70	
2P	2070	+20	39·9	48	0	8800	32	68	
7P	2030	-40	40°C	52	0	8400	47	53	
10P	2040	+10		18	8				
10A	2035	-5	39·9	80	30	7600	42	58	
2P	2020	-15	39·9	63	0	7200	50	50	
6P	2005	-15	40	2	0	6800	50	50	
10P	1990	-15		5	7				
10A	1955	-35		21	21				
10A	1955	0		115	43	6400	35	65	

PERIMENT III. Rabbit, buck. RABBIT 3. Mark Cage 22^2

HR.	WGT.	GAIN IN WT.	TEMP.	CAB-BAGE	CORN & BRAN	LEUCO-CYTES	POLYS.	MONOS.	REMARKS.
2P	1945	-10	39·5	0	2	6200	36	64	(all food
6P	1935	-10	39·5			6400	50	50	(removed from
10P	1925	-10							(cage.
10A	1900	-25	39·2			6600	34	66	
2P	1905	+ 5	39·5	5	7	7200	38	62	do do
6P	1890	-15	39·5			5400	32	68	
11P	1885	- 5							
10A	1860	-25	39·0			10600	38	62	
2P	1865	+ 5	39·5	15	13	6000	51	49	do do
6P	1855	-10	39·5			6200	30	70	
11P	1845	-10							
10A	1845	0	39·5			4800	30	70	
2P	1850	+ 5	39·5	25	7	6400	34	66	
7P	1840	-10	39·2			5800	31	69	
11P	1830	-10	39·2						
10A	1810	-20	39·2			5400	31	69	
.	.	.	.	.	.	.	.	.	(3 days interval (Fed on corn (bran & cabbage (to excess.
10A	1810		40			5800	24	76	
2P			40			10200	45	55	

(Quite healthy.
(very excitable
(rabbit.

CONCLUSIONS AS REGARDS EFFECT OF FEEDING &C., ON LEUCOCYTES OF RABBIT.

The tame rabbit is a very irregular and capricious feeder, & its intestinal capacity is very great. I have seen the stomach of a rabbit half filled with food 48 hours after a feed, although it had been absolutely starved in the interval. The glycogenic change in the liver cells, further is extremely well marked 24 hours after feeding on turnip, which shows that active assimilation is still going on then. Joseph (1909) (95) too has shown that in rabbits taken at random from a laboratory stock, the weight on the average of the stomach and caecal contents is about 10% of the body weight, the lowest % being 4%, the highest 19%. For these reasons, I did not try to find out the variations in the Leucocytes in the rabbit when fasting, and fed, as one would have done with a more regular feeder such as the cat or dog, but I endeavoured to find out as a control basis, what influence on the leucocyte count, exaggerated laboratory feeding conditions would have: and the experiments recorded above were done for this purpose. In the first place the rabbits were given

a/

a limited quantity of corn and bran and cabbage, freedom being given them to eat at any time. Next they were allowed to eat as much as they could between 11 a.m. and 1 p.m. food being withheld for the rest of the day, and lastly they were given a large excess of food with freedom to eat it when they liked.

From the tables it will be seen that the greatest variation in any of the rabbits between a minimum and a maximum estimation of the leucocytes, is less than 5,000. These variations are not related to any concurrent change in the feeding, nor have they any relation to any particular time of day. Besides if we consider the experimental error in the case, it is practically impossible for one to count a large square of the Zappert slide within 3 on either side of a mean at different estimations. This would imply within 600 per cmm. on either side of a mean and for any conclusion to be drawn with justice, at least 5 times this error on either side of a mean would have to be allowed. This would be equivalent to 3,000 per cmm. variation about a mean. To allow for experimental error in the rabbit therefore, I have come to the conclusion, that before one can say that a leucocytosis or a leucopenia/

leucopenia is present one must have a variation about a mean of at least 3,000. I have found that this is a just allowance by estimations on numerous other rabbits, some recorded in this paper and others not so recorded. The above experiments, therefore, show neither leucocytosis nor leucopenia.* This result in no way affects the question of the existence of a digestion leucocytosis, which has been shown to exist by Hofmeister (1887) (89) Pohl (1889) (155), Goodall Gulland and Noel Paton (1903-05) (76) and Erdely (1905) (56) and this for the reasons given above.

With regard to the differential counts, a marked variation in them is shown by the figures given above, but this again has no relation to food or time of day. It is extremely difficult to draw any conclusions from the differential count in experimental conditions owing to the great variations possible under normal conditions. For instance in Rabbit 2 above, on the 16th June the differential count estimated from 5 slides made at the same time (to test the accuracy of the method for estimating the differential count given above).

The/

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* For in none of the 3 rabbits experimented on as will be seen is there a variation about a mean at all approaching to 3,000.

The 1st slide showed	46%	polymorphes &	54%	mononuc.
2nd " "	42%	"	58%	"
3rd " "	40%	"	60%	"
4th " "	41%	"	59%	"
5th " "	42%	"	58%	"

while on the 17th June, the differential count was,

18th	24%	76%	and on
18th	28%	72%	

Again in rabbit (10) given afterwards the differential count on April 30th was 51% Polymorphs 49% Monos. while on May 5th it was 37% Polymorphs 63% Monos. So that it is extremely difficult to draw any conclusions from a differential count in rabbits. To conclude then, there may be in the same normal rabbit, on ordinary laboratory feeding, at different long intervals wide variations of the leucocyte count, absolutely and differentially. As regards different rabbits, my observations* confirm those of Brinkerhoff and Tyzzer, Holmes and Lisin, that in perfectly normal rabbits the total leucocyte count may vary from roughly 4000to 15,000, that usually in rabbits the mononuclears are in excess, but that in a certain number of them, the polymorphs are the more numerous.

What effect on the leucocytes of a rabbit has the injection of washed ox blood (1), intravenously, (2) intraperitoneally, (3) subcutaneously?

INTRAVENOUSLY/

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* On a very large number of ordinary laboratory Rabbits.

I N T R A V E N O U S L Y.

ravenously.
ENT I. Buck rabbit = rabbit 4. Black cropped forehead.

HOUR	LEUC.	REMARKS.	DATE	HOUR	LEUC.	REMARKS.
2P	11200		21	2P	10200	----- (3cc O.B.C.+2cc sal- (ine intravenously (on 20th at 2·30.
10A	15700		22	2P	9200	
2P	11800		23	2P	8000	
7P	10200		24	2P	9300	----- (I.B. present.
7P	8700		25	2P	10800	
10A	9900		29	9A	8800	
1P	11900		30	2P	10800	
10A	12700					
7P	10800					
2P	10600					
10A	10700					
7P	8900					
10A	11500					

MENT II. Buck rabbit. Rabbit 5, Blue and white.

HOUR	LEUC.	REMARKS.	DATE	HOUR	LEUC.	REMARKS.
2P	8400		20	10A	11300	(3cc O.B.C.+2cc saline
9A	16900		21	10A	7800	(intravenously at 2·30
2P	9800		21	7P	10300	(on 20th.
7P	8400		22	10A	8800	
7P	10200		23	10A	8800	(I.B. present.
10A	12000		23	7P	10000	
2P	12200		24	10A	9400	
9A	11600		25	10A	8500	
6P	8600		26	10A	8500	
2P	10700		27	10A	9500	
10A	12600		28	10A	11300	
7P	9400		30	10A	9900	

PERIMENT III. Rabbit, buck. RABBIT 6, white green forehead.

HOUR	LEUC.	REMARKS. DATE	HOUR	LEUC.	REMARKS.
10A	11800	23	6P	8900	---------(I.B. in blood.
3P	8000	24	10A	9800	
8P	9000	24	7P	9600	
8P	9700	25	6P	11300	
10A	9700	26	10A	8400	
2P	10000	27	2P	9000	
10A	8800	28	3P	8500	
7P	8700	29	2P	7600	
3P	9300	30	10A	10200	
10A	11100	Mar.2	2P	10000	
7P	10400				
10A	11100	(3cc O.B.C.+			
7P	10000	2cc sal.			
7P	9000	intraven. at			
10A	8400	2.30 on 20th.			
6P	8600				
10A	9300				

ENT IV. Rabbit, buck. RABBIT 7, white cropped rump.

HOUR	LEUC.	REMARKS. DATE	HOUR	LEUC.	REMARKS.
10A	13800	22	10A	10800	
2P	10400	22	6P	8600	
7P	8200	23	10A	8600	-----(I.B. present.
7P	11300	24	10A	10800	
10A	15400	24	6P	8100	
2P	12000	25	2P	10800	
10A	11700	26	10A	10000	
7P	9900	28	10A	10200	
2P	10500	30	2P	9900	
10A	13900				
7P	9400				
10A	13800	(3cc O.B.C.+2cc saline intra- venously at 2·30 on 20th.			
10A	10600				

EXPERIMENT V. Rabbit buck. RABBIT 8. Cage 23

HOUR	WT.	RECTAL 1°	LEUC.	POLYS.	MONOS.	REMARKS.
10A	2430	39·2°	7300	69	31	
12A	2430	39·2	7600	37	63	
12A			9400			(bled 3cc from ear vein.
1.30P						(Injected intravenously 3
						0.B.C. + 2cc saline.
3P		39·9	9600	50	49	
2P	2460	39·2	10000	38	62	
11A			8500	22	78	
10A		39·7	8400			
10A	2690		7500			

EXPERIMENT VI. Rabbit buck. RABBIT 9.

HOUR	WT.	CHANGE IN WT.	LEUC.	POLYS.	MONOS.	RECT. 1P	REMARKS.
10A	1745		6400	40	60	39.2	
10A	1715		7800	18	82	39.2	
4P	1705	-10	6200	20	80	39.2	
10A	1660	-45	8000	18	82	39·9	
3P			7800	28	72	39.9	
10A	1670		9200	16	84	39.2	
2P	1780	+110	10800	28	72	39.2	(7cc 0.B.C.+3cc
6P	1800	+20	10800	30	70	39.1	saline intraven-
10A	1780	-20	9400	29	71	39.1	ously at 3p.m.
2P	1810	+30	7800	23	77	39·2	of 7th.
7P	1860	+50	10600	28	72	39·5	
10A	1830	-30	8600	23	77	39.1	
2P	1840	+10	9400	25	75	39.2	
6P	1870	+30	9200	20	80	39.5	
10A	1850	-20	8200	19	81	39·2	
2P	1845	- 5	8400	35	65	39.9	
7P	1860	+15	9000	30	70	39.5	
10A	1850	-10	7400	20	80	39.1	
10A	1900	+50	10700	18	82	39.0	
2P	1900	0	8000	17	83	39.0	
6P	1900	0	10200	15	85	39·0	
10A	1880	-20	9400	22	78	39.2	
2P	1880	0	9800	25	75	39.0	
10A	1900	+20	10400	27	73	39·2	
2P	1880	-20	8400	16	84	39.1	
11A	1875	-5	9800	18	82	39.1	
12A	1940	+65	10200	19	81	39.2	
12A	1885	-55	9200	20	80	39.2	

MENT VII. Rabbit buck. RABBIT 10. Cage 28 Angora cropped head.

HOUR	WT.	RECTAL 1°	LEUC.	POLYS.	MONOS.	REMARKS.
11A	2065	40°C	8000	51	49	
11A	2080	40°C	8700	37	63	
1P						(Bled 4cc.
1.10P						(Injected intravenously
1·30P		40·1C	9200	47	53	with 2cc O.B.C. washed
10A		39·5	8400			+2cc saline.
10A		39·9	8800			
10A		39·8	8200			
10A		40·5	8600			
10A	2270	40·1	10600	40	60	
10A	2285	40·5	7800	34	66	

MENT VIII. Rabbit white buck. RABBIT 11. Cage 27, white buck.

HOUR	WT.	RECTAL 1°	LEUC.	POLYS.	MONOS.	REMARKS.
2P	1785	39·2	10000	58	42	
10A	1755	39·2	9200	43	57	
.15P						(Bled 4cc
.30P						(2cc O.B.C. + 2cc saline intravenously
·30P		40.5	9900	75	25	
10A		39·8	8600			
10A		39·8	8400			
10A		39·5	9400			
10A	1910	39·2	7400			
10A	1880	39·5	7200			

IMENT IX. Rabbit buck. RABBIT 12. Black cropped forehead, Cage 25.

HOUR	WT.	RECTAL 1°	LEUC.	POLYS.	MONOS.	REMARKS.
2P	2670	39·2	9000	58	42	
2P		39·2	7400	55	45	
3P			7200	51	49	
10A	2675	39·0	8900	64	36	
1·30P						(2cc O.B.C.+2cc saline
2P		39·2	8400	59	41	intravenously.
10A		39·5		59	42	
10A		39		51	49	
10A		39	8000	56	44	
	2730	39·2	9600	72	28	

2 INTRAPERITONEALLY.

IMENT I. Rabbit buck. RABBIT 13. Small angora cage 31.

HOUR	RECTAL 1°	WT.	LEUC.	POLYS.	MONOS.	REMARKS.
10A	39°	1420	11200	24	76	
10A	39	1410	9000	40	60	
10A	39	1460	8800	38	62	(2cc O.B.C. intra-
11A				42	58	peritoneally at 10·30
10A	39		11000	51	49	(No local pain and no
10A	39		8200	33	67	swelling.
10.30A	39	1480	7600	31	69	"
10A	39		10600	34	66	"
10A	39	1440	7400	31	69	"
10A	39		10000	31	69	"
10A	38·7		7400	32	68	(I.B. present in
10A	39	1380	7400	55	45	serum.
10A	39		7800	33	67	

(A second rabbit was injected intraperitoneally, but it developed hoea so that it was not kept under observation further).

3. SUBCUTANEOUSLY.

MENT I. Rabbit buck. RABBIT 14. Cage 30.

HOUR	WT.	RECTAL 1º	LEUC.	POLYS.	MONOS.	REMARKS.
10A	2130	39·2	7800	35	65	
1P	2117	39·1				(2cc O.B.C. subcutane-
10A		39	8200	31	69	ously at 2·25.
10A		39·2	9600			(No local swelling.
10A		39·7	7200			do.
2P		39·0	6900			do.
10A		39·0	9200			do
10A	2145	40	9700	39	61	(I.B. present.
10A	2140	39	8600			
10A	2260	39·5	8100	28	72	
10A	2145	39.2	9000	27	73.	

MENT II. Rabbit buck. RABBIT 15. Cage 29

HOUR	WT.	RECTAL 1º.	LEUC.	POLYS.	MONOS.	REMARKS.
10A	2182	39.5	7800	30	70	
10A	2205	39·2	6000	52	48	
12A						(subcutaneously 2ccOBC
11A		39·2	8100	51	49	(No pain & no swelling
10A		39·2	8700			do
10A		39·5	7600			do
2P		40·2	7100			do
10A		39·2	9100			do
10A	2215	39·2	9000			(I.B. present.
10A	2222	40	8000	32	68	
10A	2185					
10A	2235	40·2	8600	34	66	

EXPERIMENT III.

Rabbit used is rabbit 1, which was used previously for estimating the effect of food variation on the leucocytes. The rabbit was given while these observations were being made, corn bran and cabbage in excess.

HOUR	WEIGHT	RECTAL 1°	LEUC.	POLYS.	MONOS.	REMARKS.
10A	2300	39·6	10200	22	78	
2P			7800	17	83	
10A	2350	39·6	10200	17	83	
11A						(8cc O.B.C. injected subcutaneously 2cc in 4 different places.
2P	2470	39·8	8600	19	81	(No swelling, and no
6P	2510	39·8	8400	36	64	pain.
10A	2510	39·8	9400	29	71	
2P	2510	39·0	7600	30	70	
7P	2545	39·3	9200	15	85	
10A	2520	39·8	10000	10	90	
2P	2510	39·3	7800	16	84	
6P	2550	39·3	6200	30	70	
10A	2540	39·1	10000	26	74	
2P	2540	39·4	7600	21	79	
7P	2550	39·1	8400	20	80	
10A	2530	39·2	8800	14	84	
10A	2580	39·5	9200	22	78	
3P	2570	39·5	8200	19	81	
6P	2560	39·1	8000	26	74	
10A	2580	39·1	9800	13	87	
3P	2560	39·0	8200	23	77	
10A	2625	39·2	10200	18	82	
2P	2600	39·2	9200	10	90	
11A	2645	39·0	9200	12	88	
11A	2635	38·9	7800	16	84	
12A	2585	38·9	8200	14	86	

CONCLUSIONS AS TO WHETHER OR NOT A LEUCOCYTOSIS IS PRODUCED BY INJECTION OF OX BLOOD CORPUSCLES INTRAVENOUSLY, INTRAPERITONEALLY OR SUBCUTANEOUSLY.

These experiments show that the injection intravenously, intraperitoneally or subcutaneously into the rabbit of washed ox blood corpuscles does not produce a leucocytosis. As regards the differential counts duly taking into consideration the great amount of variation in the normal rabbit, one might risk the statement that such injections produce no effect on them;but it is extremely difficult to give any definite opinion. Bulloch found as mentioned above, that the immune body was formed by the mononuclears. In no sense was he entitled to draw this conclusion as his leucocyte counts were not outside the variations in the normal rabbit. The same remark holds for Holmes;and for Hunter in certain of his experiments dealing with the injection of albumen. Michaelis and Oppenheimer and Fleischmann and Michaelis (loc. at) find a leucocytosis after injection of albumens, but Dr. W.M. Scott working in this laboratory on anaphylaxis and precipitins has been unable to obtain any leucocytosis in a rabbit/

rabbit after 3 separate injections of 5cc ox serum intraperitoneally. The leucocytes were counted at short intervals during the whole period of the experiment. He very kindly allows me to make use of this result. Even if there were a leucocytosis, in no sense is it legitimate to argue from it to the leucocytes being the seat of formation of antibodies. Many substances such as Cinnamic and nucleic acids, pilocarpine &c., when injected, cause marked leucocytosis without any antibody formation. As a matter of fact a positive leucocytosis after the injection of an antigen is of no value in determining the seat of formation of the antibody, while the absence of a leucocytosis on the injection of an antigen, is strong presumptive evidence of the leucocytic organs not being the seat of formation of the antibody.

CONCLUSIONS AS REGARDS EFFECT OF EXTIRPATION OF ORGANS&c,ON THE ROLE OF THE LYMPHATIC ORGANS IN THE FORMATION OF IMMUNE BODY.

From the experiments in this section dealing with the extirpation of organs and the reaction of bone marrow and lymphatic apparatus, I conclude/

conclude, that so far as these methods can settle such a question, neither the kidney, the thyroid, the spleen, the bone marrow nor the lymphatic apparatus is the seat of formation of the Immune body against ox blood corpuscles.

EXPERIMENTS ON THE FEEDING OF RABBITS WITH OX BLOOD.

Can Immune body against ox blood corpuscles be produced by feeding Rabbits on ox blood?

It will be seen from the literature given above, that in a great many cases antibodies have been produced by feeding animals on an antigen. But there are one or two discordant results and for this reason I thought it worth while to try the effect of feeding rabbits on ox blood. It is an important thing to exclude animals with normal antibodies to ox blood, more especially with normal lysin. All the rabbits used were carefully tested to exclude a normal lysin, agglutinin, and precipitin for ox blood. (The test was made three times with three different bloods, and on three different days. For the lysin 1cc serum of the rabbit was added to 1cc 1% O.B.C. and the mixture kept at 37°C for 2 hours for/

For the agglutinin, 1cc heated serum of the rabbit was added to 1cc 1% O.B.C. Kept in incubator for 2 hrs. For precipitin ½cc of the rabbits serum was added to ½cc of 10% dilution of serum, and this was kept at room 1° for 2 hrs.*) The rabbits were then fed on 2 oz. ox blood every morning, the ox blood being simply stirred up amongst their corn and bran. After a few days they ate the mixture quite greedily.

EXPERIMENT I.

Rabbit brown doe, tested as above for normal lysin, agglutinin, and precipitin from December 6th - 8th. Feeding commenced on December 10th. Tested January 15th. Immune body, agglutinin and precipitin to ox blood found to be present.

EXPERIMENT II.

Rabbit muscovy buck, tested as before December 6th - 8th. Feeding commenced December 10th. Tested January 15th. Immune body, agglutinin and precipitin present to slightly greater degree than in brown doe.

EXPERIMENT III.

Rabbit brown rough. Tested 29th to 31st January. Feeding commenced January 31st February 15th No/

* The precipitate was then examined microscopically for agglutination.

no antibodies present as yet. February 21st Immune body, agglutinin and precipitin present.

EXPERIMENT IV.

Rabbit brown rough, eosin, tested as before from 29th to 31st January. Feeding commenced January 31st. All antibodies found in serum on February 15th.

CONCLUSIONS AS REGARDS FEEDING RABBITS ON BLOOD.

These experiments show that antibodies can be produced to ox blood by feeding rabbits on ox blood. Another thing that was found in these experiments was that where a serum was weakly lytic, the agglutinin and precipitin actions were correspondingly weak, and vice versa. Such experiments as these bring antibody formation into line with ordinary digestive and assimulative processes, and form presumptive evidence for the organs concerned in ordinary digestive and assimulative processes being also concerned here. The liver is usually accredited with a special role in these processes, and we would take it that such experiments as those above given are some slight evidence for the liver being a seat of formation of Immune body.

SECTION II.

FACTS BEARING INDIRECTLY ON THE SEAT OF ORIGIN OF COMPLEMENT.

LITERATURE.

DO SUBSTANCES PRODUCING A LEUCOCYTOSIS CAUSE AN INCREASE IN COMPLEMENT.?

Bulloch (1901) (24) stated that he found on the injection of cinnamic acid, an increase in complement going parallel with a polynuclear leucocytosis, and he argues from this that the polynuclears are the seat of formation of the complement. Falloise and Dubois (1904) (60.) show that there is no relation between the richness of the blood in leucocytes and the haemolytic and bactericidal powers of the serum coming from this blood. Busse (1908) (25.) found with substances producing leucocytosis such as nucleic acid, and "hetol", that there was no relation between the leucocytosis and the increase of complement.

DOES/

DOES PLASMA CONTAIN COMPLEMENT? ESPECIALLY DOES PARAFFINED PLASMA CONTAIN IT? AGAIN, DOES THE COMPLEMENT CONTENT OF A SERUM INCREASE THE LONGER IT STANDS IN CONTACT WITH THE CLOT?

Ascoli (1902) (5) obtained plasma by paraffined tubes from a dog which had been injected with rabbits corpuscles. On injecting 2-5cc of this into a rabbit, he got haemoglobinuria, and concluded that in vitro and in vivo plasma acts the same as serum. Doemany (1902)(46) by receiving blood into paraffined tubes, and thus preventing, to a great extent, coagulation, found that the plasma was as haemolytic as the corresponding serum. Gengou had already found (1901)(75) by this method that the plasma was not bactericidal at all, and concluded that the leucocytes were the seat of origin of alexin, giving it up on coagulation. Sweet (1903) (179) worked with cooled centrifuged paraffined plasma and found that the complement content of the plasma was as great as that of the serum. Hewlett (1903) (86) obtained goose plasma in paraffined vessels, and found that the plasma was as haemolytic as the corresponding serum. Walker (1903) (189) found that/

that the serum of rabbits contained more alexin the longer it stood up to 6 hours, but if left in contact with the blood clot for more than 6 hours, the bactericidal power decreased. Defibrinated blood contains, according to Walker, immediately after the fibrin is thrown out, its maximum of alexin, which from hour to hour decreases, and which is always greater than that of serum obtained by ordinary coagulation. He concludes that the amount of complement present in a given serum varies continuously from hour to hour after the blood is shed. It undergoes a steady increase during the first few hours, if the serum be left in contact with the clot, and only subsequently begins to show progressive diminution. Serum removed from the clot containing vessel and whipped blood, on the other hand, show no such increase of their complement which undergoes a steady diminution from the first. The complement is a leucocytic product only appearing in blood plasma, or serum as the result of a disintegration of leucocytes. Falloise (1903) (57) isolated tubes of vein in the horse, dog, rabbit and hen. He centrifuged them and pipetted off the supernatant plasma. He found that in the dog, rabbit and horse this plasma was as haemolytic as the serum, while in hens it was if anything greater. Again he drew birds/

birds blood into paraffined tubes, centrifuged and found the same result. He also prepared plasma by means of peptone, leech extract, and oxalate, and found the complement as rich in the plasmas as in the corresponding sera. He concludes that the haemolytic alexin must circulate in the blood, and that its presence in the serum is not due to destruction of leucocytes taking place at the moment of coagulation of the blood. Lambotte (1903) (101) used the same methods as Falloise, but tested the plasma and sera on micro-organisms and found the same results, and came to the same conclusions. Hermann (1904) (85) used a modification of Fredericq and Hewlett's vein method. He hung the vein up in salt solution while centrifuging. He found that the plasma of fowls so obtained did not lyse rabbits corpuscles, while the corresponding serum did. He also found that the plasma of guinea pigs, immunised with rabbit corpuscles, did not lyse rabbits corpuscles. He argues consequently to the absence of alexin in the circulating blood.* Falloise (1905) (58) undertook this research to combat some of Metchnikoff's objections to his previous work. Metchnikoff, at the congress of Hygiene at Brussels in 1903, objected that/

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* and consequently to its formation on clotting from the leucocytes.

that the prolonged centrifugation of the blood would of itself even in paraffined tubes and ligatured veins destroy a large number of leucocytes and thus set free complement. Falloise tested the effect of centrifuging for various times, on the complement content of blood drawn into paraffined tubes. He also did this experiment with isolated veins, and found in both cases that prolonged centrifugation does not increase the haemolytic power. He found also that the leucocytes are not lessened in number, nor are they affected in their vitality by prolonged centrifugation. Again he obtained a vein preparation from a horse, but instead of centrifuging it, he allowed it to settle. He found that the plasma obtained thus contained a little more, if anything, complement than the corresponding serum. Again in a similar preparation allowed to stand, he ligatured off the 3 layers (1) Red blood corpuscles, (2), White blood corpuscles, (3) plasma. He found the complement the same in each of the 3 layers. Falloise concludes that plasmas obtained by paraffined tube or ligature vein methods prepared with the minimum of leucocytic alteration, are as haemolytic or even more haemolytic (birds) than the serum which comes/

comes from the same blood drawn without any precaution after having been subjected to defibrination, and centrifugalisation. The richness in alexin of the fluids, serum or plasma, in no way corresponds to the degree of alterations of the leucocytes. The centrifugation even prolonged of cooled blood, does not destroy the leucocytes. It does not alter them at all, or very little, for they maintain their amoeboid movements, and do not give off fibrin ferment. The richness in alexin of fluids in no way corresponds to the number of leucocytes they contain. The haemolytic alexin (complement) in the plasma, in vitro cannot come then from leucocytes. It exists in the circulating blood. Lambotte and Stiennon (1905) (102) to rebut Metchnikoff's criticism at the congress of Hygiene in Brussels in 1903 that centrifugation of itself would cause phagolysis even if the blood were obtained in isolated vein or in paraffined tubes, undertook elaborate experiments to test the effect of centrifuging and change of temperature on the leucocytes. Their operations were conducted in a room kept constant at 37°C and all the manipulations were conducted in vessels, pipettes &c., heated to 37°C. They found that centrifuging/

centrifuging and temperature had practically no effect on the leucocytes within very wide limits. The vitality of the leucocytes was tested by their power to phagocyte anthrax. They found further that the liquid of an exudate deprived of its cells has the same bactericidal.and haemolytic power as the serum of the blood plasma, and further that the extracts prepared by Buchner's method from living leucocytes have no complement which could activate inactivated haemolytic sera. They conclude,therefore, that complement exists as such in circulating blood. Henderson Smith (1906) (177) found that the serum first separating from the blood may differ in its complement content from that separating later. With normal rabbit blood, the amount of haemolytic complement contained in the first serum to separate is comparatively small and progressively increases in the serum separating later for a period of 5-7 hours in the case of the rabbit. Even after removal from the clot, the amount of complement may increase in serum which separated soon after the blood was drawn, but this increase is slight. This increased activity of serum is not peculiar to the haemolytic complements. Rudolf Schneider (1908) (171) found in contradistinction to Walker that clotted/

clotted serum contained more complement than the defibrinated stuff. He found also in opposition to Walker and Henderson Smith, that the haemolytic or bactericidal complement did not increase with standing up to 8½ hours. That further temperatures had no effect on the complement content, as serum obtained at 38°C and 0°C had the same complement content. He finds that the complement is greater when the serum is soon removed from the clot, and explains this result by the clot absorbing the complement and producing autolytically, anti-complementary substances. as Buchner and Petterson assert. Schneider found further that there was no injury to leucocytes on whipping the blood or in clotting. Schneider further found taking as his criterion of change in the formed element, the production of the substance "antrhacocidin", (a thermostabile anthrax destroying substance given off by the leucocytes and blood platelets, Gruber and Futaki (1907) (69)) that fluoride plasma and paraffined plasma, in which no "anthracocidin" exists and consequently no injury to blood platelets or leucocytes has happened, contain as much haemolytic complement as the corresponding serum. Again fluoride plasma contains no fibrin ferment and contains as much complement as the corresponding/

corresponding serum. He further showed that citrated plasma contained as much complement as the corresponding serum. The same held good for leech and paraffin plasma. He concludes therefore that complement is not formed from the leucocytes on coagulation, but that it exists circulating preformed in the blood.

WHAT EFFECTS HAVE ANTICOAGULANTS ON THE COMPLEMENT CONTENT OF THE NON COAGULATED BLOOD? DO THEY PREVENT THE APPEARANCE OF THE COMPLEMENT IN NON COAGULATED PLASMA?

Nissen (1889) (139) was the first to attempt to prove the pre-existence of alexin in the circulation by means of anticoagulants. He injected a dog intravenously with peptone, and found the incoagulable blood quite bactericidal. Sawtchenko (1897) (165) found that the bactericidal power of leech plasma of the rat was the same as that of the serum, and so thought that the complement circulated free in the plasma. Mioni (1903) (131) states that he obtained ox and dog plasma entirely free from complement by treating the blood with fluoride of soda. Sodium

He/

He concludes that the normal plasma of the circulating blood of the ox, and probably of the dog, is completely devoid of haemolytic complement for guinea pigs corpuscles. The complement is only formed in vitro from the leucocytes of the blood. Falloise has repeated Mioni's experiments in the dog, ox, cock and rabbit and has absolutely failed to get his results. Petterson (1902) (145) working with K. and Na. oxalate and K. citrate plasma, found the plasma as bactericidal as the serum, & believed that the alexin circulates free in the blood, but that it is a secretion from the leucocytes in vivo. Löwit and Schwarz (1903) (120) experimented with magnesium sulphate sodium chloride oxalate, fluoride, phosphate & citrate: leech extract and iron plasmata of rabbits, guinea pigs, dogs, cats, monkeys, and also with duck and goose plasmata. None of these plasmata were found to be free from fibrin ferment. In the majority of these, they found the alexin to be present in the plasma in as great amount as in the corresponding serum. But owing to the presence of fibrin ferment in them all, they state that it is impossible for them to argue from their experiments to the pre-existence of the alexin in the circulating plasma. They express doubt as to whether the method of/

of testing extravascularly is the right method to decide as to the presence or absence of alexin in the circulating blood. Pfeiffer (1904) (151) found that in hens and geese, peptone injection decreased the haemolytic power against foreign red blood corpuscles, but did not affect the clotting power. In rabbits, peptone injection had no effect on clotting, and no effect on the haemolytic action.

IS COMPLEMENT FIRST PRODUCED BY "PHAGOLYSIS"*; OR DOES IT PRE-EXIST IN THE CIRCULATING PLASMA? (1)

Metchnikoff (1895-6) (127) stated that extra cellular destruction of bacteria and tissue cells in the peritoneum only occurs when there is "phagolysis" This phagolysis and the corresponding extra cellular destruction can be prevented, he states, by a preliminary injection of bouillon &c. He stated that if he slowed the circulation and produced an oedema, without leucocytes, in an animal immune against cholera, on the injection into this oedema of cholera vibrios, there was no Pfeiffers phenomenon*, because there was no complement here owing to the absence of leucocytes. (2) Bordet (1895) (15) confirmed Metchnikoff's experiment/

* (1) Phagolysis means according to Metchnikoff, a shedding out of strictly intracellular principles by the cell owing to it receiving some injury. He maintained that normally destruction of bacteria and tissue cells only took place inside the cells (leucocytes) by the process of phagocytosis.

* (2) Pfeiffer found that on injection of cholera vibrios into the peritoneal cavity of a guinea pig immunised against cholera that the cholera vibrios were transformed into many irregular shapes in the process of destruction which took place.

experiments with oedoema fluid in vitro, and found Immune body in oedema fluid, but not complement. Gruber and Durham (1894) (77) state that the disappearance* of the leucocytes from the peritoneal fluid is not due to their destruction, but due to their clumping on the peritoneal surface. Further, they are not polynuclears, but mononuclears, and according to Metchnikoff, it is the polynuclears that produce the bactericidal stuff. There is also a great disproportion between the large amount of alexin and the small number of leucocytes in the peritoneal fluid. According to Gruber and Durham, alexin is free in the blood serum. Pfeiffer (1896) (150) repeated Metchnikoff's original experiments with every precaution and could not confirm his results. He could not find that the injection of bouillon prevented the extracellular destruction of the organism, and he concludes that alexin is free in the peritoneal fluid. Salimbeni (1897) (164) vaccinated animals against cholera, streptococcus, and diphtheria, and confirmed Metchnikoff's views. Cantacuzene (28) (1897) working with goose spirillum, confirmed Metchnikoff's view. Wassermann (1901) (191) produced an anticomplement which prevented lysis of goats corpuscles by rabbits serum, by injecting guinea pigs with/

* Metchnikoff had advanced the view of "Phagolysis" because he found that after an intraperitoneal injection of a bacterial emulsion &c, the leucocytes disappeared from samples of the peritoneal fluid which he drew off. He thought the leucocytes had been destroyed or Phagolysed: and in this phagolysis had given off their intracellular principles.

with rabbits' leucocytes. He argues that complement is free in the plasma, and that a source of the complement is the leucocytes. Ascoli and Riva (1901) (4) produced an anticomplement by treating rabbits with leucocyte and lymph glands extracts. Levaditi (1901) (107) injected cholera vibrios into the peritoneal cavity and subcutaneous tissue of normal and immunised animals. He concludes from what he observed that the plasma of immunised animals does not contain complement and that phagocytosis takes place in these animals before any visible intervention of the humours. Sawtschenko (1902) (166) found on injection into guinea pigs of rabbit serum from rabbits treated with guinea pigs' corpuscles, haemolysis only when active serum was injected. When inactivated serum was injected there was only phagocytosis, corresponding with this the red blood corpuscles of the guinea pig injected into the peritoneum of guinea pig were phagocyted more quickly when anti-serum was added.

IS COMPLEMENT (IMMUNE BODY AND OTHER ANTI-BODIES) [K] PRESENT IN THE AQUEOUS HUMOUR OF THE EYE?

Metchnikoff (1895) (126) found no breaking up of cholera vibrios in the anterior chamber of the eye of immunised guinea pigs, because, as he says/

[K] Immune body and other antibodies are introduced here for convenience of treatment.

says, there were no leucocytes present there and consequently no complement. Achard and Bensaude (1896)(/) found agglutinin present in the aqueous humour and in the clear fluid of a hydatid cyst of rabbit which had been immunised against typhoid. Widal and Sicard (1896) (196) got agglutinin in the aqueous humour of rabbits immunised against typhoid,and also in the fluids of blisters. He got no agglutinin in the aqueous humour of three patients who died of typhoid. Levaditi (1901) (107) found the normal,and the aqueous humour,obtained by new formation,after puncture of the anterior chamber of the eye,as free from alexin as the plasma of the circulating blood. If he injected alexin in the form of active serum into the blood stream,the alexin was found to have gone over into the aqueous humour. Kemer (1901) (160) found in abrin immunity, that the aqueous humour contained antibody. Wessely (1903) (194) found that in the normal aqueous humour of animals treated with blood cells,the haemolysin did not appear, but that after puncture of the anterior chamber, after the effect of various stimulants, such as heat, chemicals, electricity, the haemolysins appeared in the aqueous humour. In contrast to the haemolysin, the agglutinin, which Widal and Sicard showed, in the aqueous humour of rabbits immunised with typhoid, appeared early in the aqueous humour of animals, immunised with blood corpuscles. Von Dungern (1903) (50) found no precipitin/

precipitin in anterior chamber of eye of rabbits treated with serum, whilst it was present in large quantities in the serum of those rabbits. Sweet(1903) (179) found that the haemolytic complement was not present in the normal aqueous humour. It is contained in the aqueous humour of new formation,due to the negative pressure in the anterior chamber rendering abnormally porous the walls of the uveal tract. The haemolytic complement must, therefore, be present in the free state in the circulating blood as there are no leucocytes in the new formed aqueous humour. He definitely concludes from his experiments in this paper, that the complement circulates already formed in the blood. Falloise (1905) (58) experimented on the aqueous humour, and found that complement is lacking in the aqueous humour, not because it is lacking in the blood, but because in the physiological conditions its passage into the liquids of the eye is hindered. He found that if he punctured one anterior chamber and withdrew fluid and tested it, he of course got no complement in it. If now he injected the animal with its own defibrinated blood intravenously, he found contrary to Levaditi's findings that the aqueous humour,obtained from the other eye, contained no complement. If he examined the/

the aqueous humour of the same eye before and after injection of defibrinated blood, he got no complement in either samples. He insists on the punctures being made without bleeding. Nedden (1907) (137) working with dysentery bacillus in the anterior chamber of the eye, found that bactericidal substances were not present in the normal aqueous humour. Schneider (1908) (171) showed first as Sweet, Wessely and Roemer had shown, that the regenerated aqueous humour contained complement which dissolved prepared blood corpuscles, while the normal aqueous humour did not do so. The aqueous humour, withdrawn the second time (after 1 hour), clotted after withdrawal. He found further that 6 hours after the primary puncture, the fluid which had collected in the anterior chamber, was devoid of complement. He further tried to get the second puncture fluid free from fibrin ferment. He drew it into a paraffined syringe by a paraffined needle into a 6% solution of Na.Fl. This was found to have no ferment, yet while the first puncture fluid contained no complement, the second always did. He considers the complement content of the second puncture fluid good proof for the pre-existence of complement in the circulating blood, for the speed with which the aqueous humour regenerates no time is given for the leucocytes to traverse the walls/

walls and no leucocytes were to be found in the aqueous humours.

WHAT TAKES PLACE, ESPECIALLY IN REGARD TO THE COMPLEMENT CONTENT, WHEN LARGE QUANTITIES OF BACTERIA OR FOREIGN CORPUSCLES &c., ARE INJECTED INTO FRESH AND IMMUNE RABBITS.

Nissen (1889) (139) injected large amounts of coccus aquatilis and vibrio cholerae intravenously into animals and found a diminution of bactericidal power. When one special organism was injected, it caused a marked and disproportionate decrease of bactericidal power for this one organism. There was less decrease for other organisms. Bastin (1892) (8) found a marked decrease of bactericidal power on the intravenous injection of bacterial emulsions: that the decrease was not specific as Nissen had found that the decrease occurred whether living or dead bacteria were injected: that the decrease was proportionate to the amount injected, and that the blood had recuperated in 7 hours. He found further that the decrease in bactericidal power went parallel with a decrease in the number of leucocytes. Denys and Kaisin (1893) (41) confirmed Bastin's observations, and/

and found the destruction of the bactericidal power after the injection of Bac aerogenes, Coli, and Anthracis that this decrease of bactericidal power is not specific, and further that the addition of dead bacteria to extravascular blood, lessened the bactericidal power. Havet (1895) (83.) found that the partial or total disappearance of white corpuscles which succeeds an injection of microbial products, causes the partial or total disappearance of the bactericidal power and the two go along absolutely parallel. Rehns (1901) (158.) injected intravenously into immunised rabbits (V.O.B.C.) 10cc washed ox blood corpuscle suspension of same strength as defibrinated blood. He found that if this quantity were ex - ceeded the animals died with symptoms of paralysis, intense dyspnoea, and rarely convulsions. P.M. they showed sometimes haemorrhages sometimes intravascular coagulation, oftener no lesions at all could be seen. With doses of 10cc and smaller doses he got at once haemoglobinuria. The injected O.B.C. therefore, at once undergo the action of the constituents of the haemolysin. Again rabbits corpuscles were sensitised with guinea pig immune serum, were then washed free of Immune body made up to the strength of defibri- nated/

defibrinated rabbits blood and injected intravenously into normal rabbits. Less than 15cc of the rabbits own blood so treated,&so injected, has caused the death of the rabbit. If the Immune body so used does not correspond to the complement of the rabbit, any quantity can be injected without harm. The Immune body is,therefore,free in the circulating blood, and complement no less surely circulates free in the plasma. The absence of complement in oedoema fluid is explained by filtration or dialysis. Schutze and Scheller (1901) (174) injected rabbits which normally have a lytic serum for goats corpuscles, with large amounts of these corpuscles. Usually 1cc rabbits serum dissolved 3cc 5% goat blood suspension and by a calculation they got the amount of goat blood corpuscles necessary to use up the lytic power in the rabbits blood. They injected more than this quantity and found that the lytic substance in the rabbits blood was used up in the first quarter of an hour. Thus in a rabbit 1500 grammes in weight,1cc of whose serum lysed totally 3cc 5% suspension of goats corpuscles in 1 hour at 37°C, and which was injected into ear vein with 10cc defibrinated goat blood,after 10 minutes 1·5cc

of/

of its serum produced no lysis in 3cc 5% suspension of goat corpuscles. The complement has therefore been used up. They found regeneration of the complement within 2 to 4 hours, and they conclude that the lysin in normal rabbits blood for goat corpuscles is used up ¼ hour after intravenous injection of large amount of goat red corpuscles. The disappearance of the lytic action of the extravascular rabbit serum after the injection of goat blood, is to be attributed to a using up of the corresponding complement in the animal body. The reappearance of the lytic substance follows in the first 2-4 hours after the injection. Levaditi (1901) (107) injected cholera vibrios intravenously into normal and immunised animals, and found that in the immune animals so injected the vibrios are not transformed into granules in the circulating plasma, but the transformation only takes place there inside the polymorphs. Pfeiffer's phenomenon, he states, only takes place in the organs, and there it operates by means of the complement set at liberty by the phagocytes accumulated in the interior of these organs. He concludes that the plasma of immunised animals does not contain complement. Sachs (163) (1903)/

(1903) injected 30-35cc washed ox blood corpuscles intravenously into rabbits and determined the presence of the O.B.C. from time to time in the rabbits' blood by adding inactivated immune body V.O.B.C. and·3cc rabbit complement to a sample of blood drawn from the rabbit. Only as long as O.B.C. were present in the rabbits' blood did haemolysis take place in the above mixture. The O.B.C. were present in the rabbits' blood for periods varying from 46 hours to 70 and 92 hours. The disappearance of the ox blood corpuscles from the rabbits' vessels is critical as shown by the marked and sudden colouration of the urine with haemoglobin.* Sachs found, as Bulloch had done, that the immune body appeared in the blood of the rabbit after an injection of O.B.C. intravenously or intraperitoneally, first, on the third day. The first appearance of the immune body in the blood occurs simultaneously with the disappearance of the O.B.C. from it, which takes place in about $2\frac{1}{2}$ to 3 days usually. The rapid solution of the ox blood in the organism of the rabbit causes according to Sachs a marked diminution of the complement content of the blood. Von Dungern and Bulloch state that the complement is hardly or not at all affected by immunisation with blood. This, according to Sachs, was due to their drawing their conclusions/

* which takes place on or about the 3rd day when the immune body first appears in the rabbits' blood.

conclusions from the end point of the reaction, or on the other hand the intervals of estimation were so long that the critical moment might easily be overlooked. He finds in the first place a sinking of the complement, secondly a rise of it and thirdly a return to normal. Sachs considers this research to speak eloquently for the presence of complement free in the circulating blood. Here 3 days intervene between the injection of the corpuscles and the first onset of their solution, so that this would entirely avoid the phagolysis which Levaditi and Metchnikoff bring in on all occasions to explain the origin of complement. Simnitzky (1903) (176) found on intravenous injection of O.B.C. into a rabbit, a marked leucopenia but no diminution of alexine during the first 24 hours. He considered that the leucopenia was due to destruction of leucocytes, and that the alexin remained the same in spite of this was proof for his conclusion that the alexin existed free in the blood. Löwit and others find, however, that the leucopenia is due to the gathering up of the leucocytes in the internal organs. Batelli (1900) (9) injected dogs of about 10 Kilos. intravenously with sheep, pig or horse corpuscles (washed corpuscles in saline*), the emulsion being of the strength of defibrinated/

* 50cc of this suspension was injected.

defibrinated blood. The whole of this blood was dissolved within 2 minutes of injection. After the injection he found a diminution of the haemolytic power proportional to the quantity of corpuscles injected, the haemolytic power becoming nil when a large quantity of corpuscles were injected. Several hours after the injection into the dog of 50cc of this suspension, the number of leucocytes increase considerably, and there is a fall in the haemolytic power, and the haemolytic power is therefore not in accordance with the leucocytosis. The same phenomena occurred in the rabbit, except that the injection of large quantities of easily haemolysed corpuscles very often produced rapidly the death of the animal. Levaditi (1905) (112) in continuance of his work recorded above, with cholera vibrios investigated what took place when pigeon corpuscles were injected intravenously into the guinea pigs. The guinea pig's serum is normally haemolytic to pigeon corpuscles, according to Levaditi 0.5cc completely dissolving a drop of pigeons' blood. He injected into the jugular veins of fresh guinea pigs 1 to 1.2cc of a thick emulsion of pigeon corpuscles. They bore this injection quite well, while a similar injection into guinea pigs immunised against pigeon corpuscles caused great dyspnoea and extreme collapse. The respiration/

rexpiration troubles predominated. This difference between the effect in fresh and immune animals is due according to Levaditi to rapid lysis, phagocytosis and the accumulation of the phagocytes, nuclei and stromata in the capillaries of the lung. He finds that there is no extra-cellular haemolysis, but an extremely rapid and energetic phagocytosis in the spleen in the case of the fresh guinea pigs injected with pigeon corpuscles;while in the vaccinated animals there is extracellular solution due to phagolysis and much less marked phagocytosis. These differences,he holds,explain the different reaction of the two animals to the injection. Levaditi further takes his experiments with fresh guinea pigs as evidence for the non-existence of complement in the circulating blood. The guinea pig's serum is very haemolytic in vitro for pigeon corpuscles. "How comes it then" he asks, "that at the end of an hour one can find in the "guinea pig's blood unaltered pigeon's corpuscles,if "the complement is free in the plasma"? The simple explanation of this is that he injected an enormous dose of the corpuscles, much in excess of what the serum could have dissolved in the time in vitro. Trommsdorff (1906) (187) found that 2 to 3cc washed O.B.C. was the calculated amount for the total absorption/

absorption of complement in guinea pigs weighing 300 to 400 grammes. He injected this amount intravenously and found a decrease in alexine after 30 minutes, the maximum decrease being between the 3rd and 6th hour. After 9 hours the original amount of alexin had reappeared; while in animals weakened by cold and exhaustion the regeneration was delayed, so that even 24 hours after the injection the alexin content was very much below normal. Coca (1908) (36). The cause of death when foreign blood corpuscles are injected into an animal is a mechanical blocking of the lung vessels.

WHAT TAKES PLACE WHEN INTO THE PERITONEUM OF ONE ANIMAL IS INJECTED HEATED INACTIVATED SERUM HAEMOLYTIC FOR ITS OWN BLOOD CORPUSCLES OBTAINED FROM A SECOND ANIMAL* WHICH HAS BEEN INJECTED SEVERAL TIMES WITH BLOOD CORPUSCLES FROM THE FIRST ANIMAL?

Gruber (1901) (78.) injected rabbits with guinea pig corpuscles, thus obtaining a serum lytic for the guinea pig corpuscles. He inactivated their serum and found that it could be activated again by guinea pig serum. He then injected into the peritoneal cavity of fresh guinea pigs 4 to 10cc of the inactivated/

* of another species.

inactivated rabbit serum v. guinea pigs' corpuscles. This caused several hours after a marked haemoglobinuria and marked anaemia due to the inactivated immune body being absorbed into the circulation, fixing on to the guinea pig corpuscles, and with the help of the complement in the plasma, causing a lysis of the corpuscles. He concludes that the alexin circulates free in the plasma. Levaditi (1902) (//o.) criticises Gruber's experiment by saying that the introduction of the heated serum into the peritoneal cavity causes a phagolysis there and a setting free of complement, by two methods: firstly, by the simple injection,* and secondly by the heated immune serum containing a leucotoxin;so that he maintains that there is partial reactivation in the peritoneal cavity. In guinea pigs "prepared" by a previous injection of bouillon, he says there is no phagolysis but only erythro-phagocytosis, after an injection of heated serum. He maintains further, that all the signs of haemolysis in the general circulation such as anaemia and haemoglobinuria, are much more marked in "prepared" guinea pigs than in guinea pigs "not prepared", and that they are proportional to the phagocytosis in the peritoneal cavity, and inversely proportional to the phagolysis there. The immune body/

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* Phagolysis phenomenon of Metchnikoff.

body is rapidly injected into the peritoneal cavity, is rapidly absorbed, and goes to lodge by preference in the organs which normally serve for the destruction of the red blood corpuscles, in particular the spleen. The spleen, too, is the seat of an intense phagocytosis of red blood corpuscles, and these phenomena are much more accentuated in "prepared" than in "unprepared" animals, and he has even seen in the "prepared" animals phagocytosis in the blood stream. So that the intra-splenic phagocytosis is the cause of the anaemia and haemoglobinuria. Further, Levaditi took two guinea pigs A and B. He "prepared" B by giving it 10cc broth intraperitoneally 18 hours before experiment. He then gave A and B 7cc intraperitoneally of inactivated immune serum, and 4 hours after, on bleeding them to death, he prepared plasmata from each by means of paraffined tubes. He found in both that the plasmata (centrifuged immediately) were uncoloured, while the serum of the corresponding bloods obtained by ordinary coagulation were markedly haemoglobin tinted, B's serum being more haemoglobin-tinted than A's. Of course, he argues from this that the complement is formed from the leucocytes by phagolysis when clotting. He concludes that phagocytosis in the spleen is the cause of/

of the anaemia and haemoglobinuria in the guinea pigs when injected intraperitoneally with heated immune serum against their own blood corpuscles, and states that complement is not free in the circulations. One hastens to say here that not one of Levaditi's experiments or statements in this paper carries the slightest degree of conviction with it. Bellei (1904) (11) repeated Gruber's experiment and drew off samples of blood at various times after injection into paraffined tubes and for coagulation. He found both the plasma, centrifuged immediately, and the serum, after separation, haemoglobin tinged, the serum being slightly more so. The last, as Bellei showed, is due to the serum standing longer in contact with the blood corpuscles. These experiments of course, tell strongly against Levaditi's statement that the haemoglobin only appears in the drawn blood after coagulation, when consequent phagolysis and setting free of the complement has taken place. Ceaparu (1908) (34) introduced into the stomach of rabbits and dogs, sheep serum specific against the red blood corpuscles of rabbits and dogs respectively. In both the serum was absorbed and reduced the number of red blood corpuscles from 6 millions to 2 millions.

REMOVAL/

REMOVAL OF COMPLEMENT FROM A SERUM BY VARIOUS PARTICULATE NEUTRAL SUBSTANCES.

Schneider L. (1897) (170.) shewed that the alexin of the blood was destroyed by the growth products of typhoid. Von Dungern (1900) (49.) found that emulsions of organs even of the animal supplying the serum, could remove entirely the complement from that serum. Emulsions of various bacteria yeast cells etc. can also remove the complement. Wilde (1901) (197) found that contact with living and dead bacteria, yeast cells, red blood corpuscles, organ cells, insoluble albumens, especially aleurone, could destroy the bactericidal and haemolytic action of the alexin of cattle, dog and rabbit blood serum. He found also that these substances could absorb complement intra vitam. Muir (1903) (134) found among other things that the stromata of red blood corpuscles could take up a large amount of complement, and this direct union of complement with cells takes place most rapidly at 37° C. Sweet (1903) (179) produced leucocytic exudates by means of aleurone, and got extracts by means of Buchner's method. He tested the effect of these preparations on sensitised corpuscles and haemolytic mixtures. He concluded that the haemolytic/

haemolytic complement is hardly at all present in the exudate produced by aleurone in the pleural cavity, and what little complement is present is to be found in the serous portion of the exudate and is not contained in the leucocytes, whether polynuclear or mononuclear. The complete breaking up of the leucocytes in such an exudate sets free no haemolytic complement, not even when the exudate is composed of 50% mononuclears. He further obtained removal of complement from serum by filtering it through a Chamberland filter. Gruber (1903) (80) added emulsion of leucocytes to sensitised O.B.C. and found no haemolysis. Hoke (1903) (90) found that addition of rabbits leucocytes to rabbit serum, far from secreting, had absorbed the complement. He found that the emulsions of the spleen and bone marrow absorbed much more complement. Landsteiner and Stankovitch (1906) (105) shewed that complement could be absorbed by various inert substances in suspension or in colloidal solution such as kaolin, casein, glycogen and certain liquids such as cholesterin. Wassmüth (1907) (190) found that rabbit leucocytes are able to suspend, up to a certain point, the globulicidal property of rabbit's serum with regard to guinea pig erythrocytes. This property he finds belongs/

belongs to dead leucocytes as well as living ones. Neufeld (1908) (/36.) found that the addition of emulsions of leucocytes to mixtures of sensitised cells and complement prevented haemolysis, and concludes that the leucocytes neither secrete complement, nor in clotting give it off to the serum for they contain absolutely no active complement. Browning and McKenzie (1909) (21) found that a turbid emulsion could of itself deviate complement, and this had to be taken into account in the Wasserman syphilis reaction. Muir (1909) (/35) removed complement from a serum by means of filtrationthrough a Berkefeld filter.

SUMMARY AND CRITICISM OF LITERATURE.

In summarising the literature given above it will be seen that it deals with the seat of origin of complement and more particularly with the question as to whether or not the leucocytes are the seat of origin of it. This last question further resolves itself into one as to whether or not the complement is formed at the time of clotting and as the result of clotting, from the leucocytes of the blood, and as to whether or not the complement preëxists in the circulating blood. The evidence for the solution of/

of this question as it occurs in the literature can be divided into two groups, the presumptive evidence and the conclusive evidence. The prevention of the clotting of the blood by anticoagulants and the finding that plasmata so produced contained quite as much complement as their corresponding sera is presumptive evidence that the complement circulates, preformed, in the blood stream; and when further plasmata obtained by numerous anticoagulants, all having a different mode of action, all unite in the one characteristic that they contain as much complement as their corresponding sera, this presumption is greatly strengthened but it can never go the length of being conclusive evidence, For owing to the intrinsic shortcomings of the method as a method, it can always be urged that although anticoagulants may prevent coagulation of the blood, there is no way of shewing that, at the same time, they may or they may not stop an action giving rise to complement from some precursors in the blood, and which action goes parallel with, but is entirely independent of the coagulation process. With regard to the evidence of a presumptive nature adduced by Metschnikoff in support of his theory - that of the absence of complement in the aqueous humour and in the oedema fluid - this can be shewn to be no evidence at all for the aqueous humour is a highly specialised secretion from the ciliary bodies, and while the absence/

absence of complement in the oedema fluid could be explained by dialysis and selective secretion, there is doubt as to the existence of this absence. Again with regard to Metschnikoff's position that Pfeiffer's phenomenon in the peritoneal cavity is due to phagolysis and can be prevented by a preliminary injection of bouillon some 24 hours previously, intracellular destruction then only taking place, equally good investigators, such as Pfeiffer himself, have repeated Metchnikoff's experiments with every precaution, and have been unable to confirm his results. Bulloch, further, has found that substances producing a leucocytosis such as cinnamic acid produce a rise in complement and argues accordingly to the leucocytes being the seat of origin of complement. Busse performing exactly the same experiments could obtain no such result, and Falloise and Dubois have shewn that there is no relation between the richness of the blood in leucocytes and the haemolytic power of the serum coming from the blood. Besides in principle such a method can only provide the weakest of all presumptive evidence. With regard to the more conclusive evidence, two experiments stand out notably, those of Sachs and Gruber. Sachs injected a rabbit intravenously with ox corpuscles; nothing could be detected/

detected until the third day when immune body having been formed, there was a critical haemoglobinuria and haemoglobin in the serum together with the disappearance of the ox corpuscles from the blood. There was no leucocytosis, and it is preposterous to think that the so-called phagolysis would conveniently delay its appearance for 3 days until the opportune arrival of the immune body. With regard to Gruber's original experiment and his later modifications of it, in which a guinea pig is injected intraperitoneally with heated immune serum against its own blood corpuscles, and in which it develops haemoglobinuria and anaemia as the result of the absorption of the immune body into the blood stream and its collaboration there with the complement of the blood, the criticisms of Levaditi can only be characterised as the exasperating obstructionist tactics of one who is pledged to defend a cause at any cost.

Though having more reference to the previous section on the seat of origin of immune body, and to the succeeding section - that on the liver - the literature on the absorption of complement by organ emulsions and indifferent suspensions is here given. This has been done because this section deals with the complement particularly, and also because this in/

in vitro and in vivo absorption of complement may explain the results obtained by Nissen and others where a diminution of complement was obtained on the intravenous injection of large amounts of bacteria; and of Rehns and others who found that the injection of blood corpuscles into the blood stream of an animal whose serum is lytic for these blood corpuscles, causes a diminution of complement where it might be supposed that the corpuscular stromata, as Muir has shewn in vitro, would take up the complement. I shall say more in extension of this later.

All of the experiments given below, with the exception of those of Sachs and Gruber (which I have repeated) were done before I had access to the literature relating to them. It is necessary, however, to give them owing to the contradictory findings of several investigators.

WHAT EFFECT HAVE ANTICOAGULANTS ON THE COMPLEMENT CONTENT OF THEIR RESPECTIVE PLASMATA AS COMPARED WITH THE COMPLEMENT CONTENT OF THEIR CORRESPONDING SERA?

EXPERIMENT I. Leech extract: (Hirudin Sacchse) in vitro.

To/

To test first if leech extract had any influence on the complement content of a serum in a strength equal to that to be used for comparing serum and leech plasma. To 2cc serum was added 1·07cc leech extract solution 0.1 gr. in 20cc: and to 2cc of the same serum was added 1·07cc saline. The minimal haemolytic complementing dose was then estimated as follows.

haline of serum?

Complements/

Complements undiluted: 1% O.B.C. washed 6 times in ·85% saline used: heated immune serum diluted 1 in 10 with saline, of which minimal haemolytic dose for 1CC 1% O.B.C. was 0·02cc.

* 1% O.B.C.		H.I.B. diluted.		COMPLEMENT (leech, serum.)			SALINE.	
1.	1cc +	·02	+	·5	+	0 =	c	
2.	1cc +	·02	+	·4	+	·1 =	c	2
3.	1cc +	·02	+	·3	+	·2 =	c	hrs.
4.	1cc +	·02	+	·2	+	·3 =	++++	at
5.	1cc +	·02	+	·1	+	·4 =	+++	37°
6.	1cc +	·02	+	·05	+	·45 =	+	C.
7.	1cc +	·02	+	·0	+	·5 =	0	

Throughout the research c = complete: ++++ = nearly complete: +++ = good amount: + = trace: 0 = none.

A similar series was also made with the serum + saline and incubated for the same time at 37° C., the results were -

1 = c
2 = c
3 = c
4 = ++++
5 = +++
6 = +
7 = 0

* **In all** the experiments on anti-coagulants the same formula was gone through. The corpuscles were washed 6 times, and 1% suspension made up. Heated immune body was used diluted: and the titre of this immune body had been accurately determined previously. The same series of tubes was made up in each case: so that it will be unnecessary to formally record such tables in the other experiments of this division and only the results will be given.

This/

This shews that the leech extract had no effect on the complementing power of the serum. (In all the experiments detailed in this section - except where definitely stated to be otherwise - the minimal haemolytic complementing dose is the smallest dose of the serum in quesion, which when added to 1cc 1% ox blood corpuscles sensitised with a minimal haemolytic dose of heated immune body caused complete haemolysis in 2 hrs at 37° C.). Next, a rabbit was taken from the ear vein, 3½cc were bled into tube 1, then 7cc were bled into tube 2 which contained 3cc of leech extract solution 0·1 gr. in 20cc saline, then 3½cc was again bled into tube 1. Tube 1 and tube 2 were allowed to stand till serum separated in tube 1. (2hours): then both were centrifugalised, and the supernatant fluids pipetted off - from tube 1 = serum, from tube 2 = leech plasma. To 2½cc of the serum of tube 1 was added 1·07cc leech extract solution, 0·1 gr. in 20cc saline = leech serum. The leech serum and the leech plasma were now tested and the M.H.C.D. was found to be the same in each. So that leech plasma/

Hereafter M.H.C.D. will be used for the minimal haemolytic complementing dose.

plasma obtained by drawing the blood into leech extract solution contains as much complement as the corresponding serum. The leech plasma did not coagulate as long as it was observed,-for several days.

EXPERIMENT 2. Leech extract in vivo.

Rabbit: buck: weight 1180 grammes, was bled 5cc from ear vein at 10 o'clock,(tube 1,) at 10.10 it was injected intravenously with 20cc of a solution of leech extract 0·1 gr. in 20cc normal saline. The rabbit died in 5 minutes: blood was obtained from the heart, tube 2. Tube 1 and tube 2 were kept at room temperature until serum separated in tube 1 - till 12·45: tubes 1 and 2 were then centrifugalised, supernatant fluids pipetted off - from tube 2 = leech plasma, tube 1 = serum, and as this rabbit contained about 60cc blood to which was added 20cc leech extract solution, to 3cc of this serum was added 1cc of the leech extract solution = leech serum. The leech plasma and leech serum were then tested and the M.H.C.D. was found to be the same in each; so that the leech plasma obtained by injection intravenously of leech extract solution contained as much complement as the corresponding serum.

The/

The leech plasma did not clot as long as it was observed - for several days.

EXPERIMENT 3. Pot. oxalate in vitro.

It had been found previously, by actual experiment by myself, that oxalate had no effect on the complementing power of serum.

Rabbit: buck: at 10.45 bled 4½cc into tube 1, then 9cc into 1cc 10% Pot. oxalate in normal saline in tube 2, then 4½cc bled again into tube 1. Tubes 1 and 2 stood at room 1° until serum separated in tube 1 at 12.30, both tubes then centrifuged: supernatant fluid pipetted off, from tube 2 = oxalate plasma, from tube 1 = serum: to 3cc of this serum were added ·3cc 10% Pot. oxalate solution = oxalated serum. This oxalate plasma and oxalate serum were then tested and the M.H.C.D. in each was found to be the same. No coagulation in the oxalated plasma: so that oxalated plasma contains as much complement as the corresponding serum.

EXPERIMENT 4. Sod. Citrate in vitro.

It had been previously found, by actual experiment by myself, that Sod, Citrate had no effect on the complementing power of serum.

Rabbit/

Rabbit: buck: at 12.30 4½cc bled into tube 1, then 9cc bled into 1cc 10% sod. citrate solution in tube 2, then 4½cc bled again into tube 1. Tubes 1 and 2 stood at room 1° till 2 o'clock: both tubes then centrifugalised, supernatant fluid pipetted off, from tube 2 = citrate plasma, from tube 1 = serum. To 3cc of this serum was added 0·3cc of 10% citrate solution = citrated serum. Citrated plasma and Citrated serum tested, and the M.H.C.D. found to be the same in each. No coagulation in the citrated plasma: so that citrated plasma contains as much complement as the corresponding serum.

EXPERIMENT 5. Sodium fluoride invitro.

It was found that sodium fluoride in strength of about 0·60% had a fairly marked effect in destroying the action of the complement.

Rabbit: buck: at 10 o'clock 4cc blood drawn into tube 1, then 8cc drawn into 2cc 2·5% sod. fluoride solution in ·85% saline in tube 2, then 4cc again drawn into tube 1. Tubes 1 and 2 stood at room 1° till serum separated in tube 1, at 12 o'clock, then centrifugalised, and supernatant fluids pipetted off, from tube 2 = fluoride plasma, from tube 1 = serum. To 3cc of this serum was added ·75cc of the fluoride solution = fluoride/

fluoride serum. The fluoride plasma and the fluoride serum were tested and the M.H.C.D. was found to be the same in each. There was no coagulation in the fluoride plasma. Here it might be that the plasma actually contained more or less complement than the serum, and that the action of the fluoride on the plasma, being different from that on the serum, was such as to diminish or increase it, so that the M.H.C.D. of the fluoride plasma came out the same as that of the serum. This is hardly possible for the two series of 7 tubes for estimating the M.H.C.D. came out exactly the same in every respect, which is something more than a coincidence: so that we may conclude provisionally that the fluoride plasma contains as much complement as the serum.

CONCLUSIONS FROM EXPERIMENTS ON ANTICOAGULANTS.

These experiments therefore show that with leech plasma, oxalate plasma, citrate plasma and very probably with fluoride plasma the minimal haemolytic complementing dose is the same as in the corresponding serum. Such results are presumptive evidence for the existence of complement in the circulating blood and its non-formation from the leucocytes on clotting. Owing/

Owing to the extreme difficulty of obtaining paraffined plasma and of doing Frederiocq's ligatured vein experiment I did not attempt them. Besides the difficulty of their performance, they can only supply at best presumptive evidence, and again there is the probability, amounting almost to certainty in many cases, that the plasmata so obtained will coagulate as soon as removed to a non-paraffined vessel, or as soon as mixed with the test corpuscles; or if they do not do this there is the great probability that, at least in the paraffined tubes if the most exacting care is not taken, the blood coagulates unobserved and the process of centrifuging shakes the clot so formed to the bottom of the tube along with the corpuscles, the result being that one obtains in reality a serum. This at least has been my experience and that of Dr Ritchie (personal communication). Gengou also admits in his experiments that he never got plasma free from some clot.

THE NEXT EXPERIMENTS I DID WERE WITH REGARD TO THE HAEMOLYTIC COMPLEMENT CONTENT OF AQUEOUS HUMOUR, OF BLISTER FLUID AND OF TAPE WORM CYSTS IN THE RABBIT.

EXPERIMENT I.

Is complement present in normal aqueous humour?

Rabbit, killed: aqueous humour drawn with glass pipette from anterior chamber of the eyes; about ·3cc clear watery fluid obtained: this fluid added to ·2cc I% sensitised ox blood corpuscles incubated for 2 hrs at 37°C. - result = no haemolysis.

EXPERIMENT II.

Is complement present in normal aqueous humour?

Rabbit: killed: ·5cc aqueous humour drawn off with glass pipette through the cornea: this added to 0·35cc I% sensitised ox blood corpuscles and incubated for 2 hours at 37°C. - result = no haemolysis: to this tube was then added ·2cc Heated Immune Serum (the purpose of this being to demonstrate if ~~it~~ there were present the smallest trace of complement: the larger the amount of I.B. taken the smaller is the amount of complement that can be demonstrated: see page (147).) -·2cc heated immune serums/

serum, the minimal haemolytic dose of which was 0·005cc for 1cc 1% O.B.C. This was incubated at 37°C for 2 hours; result was still no haemolysis. There was then added to this tube 0·5cc complement. Result was complete haemolysis in 5 minutes. The minimal haemolytic dose of this rabbit complement was ·3cc for 1cc 1% sensitised ox blood corpuscles.

EXPERIMENT 3.

Does the aqueous humour, reformed after puncture contain complement.

Rabbit anaesthetised with ether: aqueous humour withdrawn from both eyes = 0·5cc: no trace of bleeding. This was added to 1cc 2% O.B.C. ·2cc (for same reason as in last experiment) strong immune serum. The result was no haemolysis in 2 hrs. at 37°C. The rabbit was kept under ether for an hour, and at the end of this hour 0·25cc aqueous humour was again obtained There was absolutely no bleeding, yet in ½ hour this coagulated and when added to 1cc 2% O.B.C. + 2cc strong heated immune serum/

serum there was complete haemolysis in ½ hour.

EXPERIMENT. 4

Does the aqueous humour of an immune rabbit, V.O.B.C. contain immune body.

Rabbit: blue and white had been injected with ox blood corpuscles frequently and the M,H,D, of its heated immune serum: for 1cc 1% OBC when fully complemented was ·005cc. This rabbit was killed and its aqueous humour withdrawn with great care by means of a glass pipette. There was no bleeding: 0·5cc of this aqueous humour was added to 1cc 1% O.B.C. There was no haemolysis in 2 hours at 37°C. This might be due to absence of complement or absence of immune body: therefore to the tube was added ·5cc rabbit complement and there was no haemolysis in 2 hours. at 37° showing that immune body was absent from the aqueous humour: and when to this tube a minimal haemolytic dose of immune body had been added there was complete haemolysis in 30 minutes. Agglutinin was also absent from the rabbits' aqueous humour. In this case therefore/

therefore immune body was absent from the aqueous humour.

EXPERIMENT. 5.

Done with the same purpose in view as in the last experiment.

Rabbit had been frequently injected with O.B.C. and M.H.D. of its serum for Icc I% O.B.C. when fully complemented was ·00Icc. This rabbit was killed, ·2cc of its aqueous humour and Icc I% O.B.C. gave no haemolysis in 2 hours. at 37°C. but on adding to this ·3cc of complement the haemolysis was complete in I½ hours. There was no bleeding and the aqueous humour was quite fluid and did not clot. So that in this case Immune body was present in the aqueous humour but no complement. The immune body was present in the aqueous humour in a much less degree than in the serum.

EXPERIMENT. 6.

Does the fluid from a tape worm cyst - like the aqueous humour, not contain complement.

A rabbit was found with a cyst of Taenia (coenurus)/

(coenurus) serialis below its neck: The fluid from this was withdrawn and tested for complement. No complement was found in 0·5cc of it. The fluid was watery and contained very slight traces of albumen.

EXPERIMENT 7.

Does Blister fluid contain complement?

A rabbit was blistered on the inner surface of the ear. The fluid from this was obtained quite free from blood and was clear and watery, complement was found to be present in this blister fluid.

CONCLUSIONS FROM THE EXPERIMENTS ON THE AQUEOUS HUMOUR.

From these experiments it will be seen, in harmony with Metchnikoff's experiments on Cholera that haemolytic complement is absent from the normal aqueous humour: but that the fluid which fills the anterior chamber of the eye within an hour after puncture coagulates and contains complement. This is in harmony with Schneider's findings who further found/

found that the fluid withdrawn from the anterior chamber 6 hours after puncture was again devoid of complement. From such things as these we conclude that the aqueous humour is a very special secretion of the ciliary glands, that when the aqueous humour is rapidly removed to preserve the integrity of the eye there is a rapid transudation instead of a secretion from the blood vessels and this transudation contains complement. Metchnikoff from experiments on the aqueous humour is in no sense warranted in drawing the conclusion he does, that complement does not exist free in the circulating blood, but comes from the leucocytes, no more than I am warranted in drawing a similar conclusion from my experiment on the tape worm cyst where I found no complement in its fluid. With regard to the oedema fluid and Metchnikoff's statement that it doesn't contain complement, in the first place one could say that if this was really so it could be explained by a selective transudation from the blood vessels, but on the other hand such experiments as those showing complement to exist in the aqueous humour one hour after the first puncture tell against this view. Moreover Eason (52) in his work on paroxysmal haemoglubinuria obtained his serum for working by blisters, and this blister/

blister fluid always contained complement. Again in the experiment detailed above a rabbit complement was found in the blister fluid although the fluid contained no blood, was quite clear and did not coagulate. Further Pfeiffer repeating Metchnikoff's experiment in the subcutaneous tissue with every precaution was never able to confirm Metchnikoff's results.

A further point of interest in this connection is that the ciliary bodies may entirely keep back immune body and agglutinin from the aqueous humour, even although that immune body may be present in such an amount in the serum that ·005cc when fully complemented could completely haemolyse 1cc 1% O.B.C.as shown in rabbit of Experiment 4: where the immune body is not entirely kept back, it is present in very much weaker degree in the aqueous humour as in rabbit, of Experiment 5. Metchnikoff admits that immune body exists in the circulating plasma but the use of his logic with regard to the complement would lead us to suppose that in rabbit 4 such a state of matters was not the case, moreover Metchnikoff states definitely (Immunity in Infectious diseases English trans. page 217), that fixative is not present in the aqueous humour/

humour.

Repetition of Sach's experiment: Effect of profound bleedings on the complement content of Rabbits' blood.

I shall now go on to a consideration of Sach's experiment and certain other experiments which have a bearing on it. In the experiments recorded in this section the same blood corpuscles, and as far as possible the same suspension of them were used for each separate experiment: and further the serum obtained on one day was used as a control for the corpuscles used on the next day, so that a check was always kept on the corpuscles. The corpuscles between the various estimations were kept in an ice chest at about 8°C and one batch of corpuscles was never used for longer than 4 days. The experiments generally did not last for more than 4 days. The blood was collected under the same conditions and the sera allowed to separate under so far as possible the same conditions, that is 2 hours in incubator and then about 12 hours at room 1°.

What effect has serious bleeding on the complement content of a rabbit's serum?

EXPERIMENT/

EXPERIMENT 1.

White rabbit, buck, weight 1950 grammes, bled 20cc at 4 p.m. from the ear vein on January 26th. put into cage with turnip to eat, bled 20cc again at 6·45 p.m. January 26th, that is 2½ hours after the 1st bleeding. On January 27th at 10 o'clock a.m. it was bled 15cc again it was bled 5cc at 3·40 p.m. on January 27th: on January 28th it was again bled 5cc at 11·30 a.m. and on January 29th. at 10 a.m. it was bled 6cc. The various sera obtained from these bleedings were tested with the same batch of ox corpuscles, with the same suspension of them as far as possible, and where this was impossible, when a new suspension was made up the transition was made to this new suspension by doing a double series, 1 series with the old suspension and one with the new. Besides even up to the last day of the experiment, the sera of the day were controlled by some of the serum remaining over from the previous days. In 10 estimations series of 6 tubes each, made for estimation of the complement content of the day's sera and as controls with previous day's sera and corpuscles, the M.H.D. of the complement was the same in every case, and the estimation series as regards where haemolysis commenced, where it was complete/

complete&the grade of haemolysis in the intermediate tubes were exactly the same in every respect.

I give an example of the method of estimation for January 26th 4 p.m. and 6.45 p.m.

Complements undiluted: 1% O.B.C. washed 6 times in .85% saline: heated immune serum diluted 1 in 10 of .85% saline, minimal haemolytic dose for 1cc 1% O.B.C. = .015cc:

1% O.B.C.		H.I.S. 1 in 10		COMPLEMENT 4 o'clock:		SALINE.			
1. 1cc	+	.015	+	.5	+	0	= c	)	
2. 1cc	+	.015	+	.4	+	.1	= c	)	
3. 1cc	+	.015	+	.3	+	.2	= c	)	2 hours
4. 1cc	+	.015	+	.2	+	.3	= c	)	at
5. 1cc	+	.015	+	.1	+	.4	=++++	)	37° C.
6. 1cc	+	.015	+	.05	+	.45	=++	)	
7. 1cc	+	.015	+	.0	+	.5	= 0	)	

A similar series was made up for the serum obtained at 6.45 and the results were:-

1 = c
2 = c
3 = c
4 = c
5 = ++++
6 = ++
7 = 0

(This example will serve to illustrate the method/

ethod pursued in the subsequent experiments for stimating the minimal haemolytic dose of the comple-ent). So that from this experiment it appears that rofound bleeding to the extent of 55cc - more than alf the rabbit's blood - in 18 hours has no effect on he complement content of the serum. (The amount of lood in this rabbit's body = 93cc, for method of alculation see below).

XPERIMENT 2.

Rabbit weight 2150 grammes, buck, bled 35cc, o more blood could be obtained from the ear vein of this rabbit at this sitting owing to the weakness of the circulation. From ear vein at 11·45 a.m. At 12·45 of same day it was bled 6cc. Next day at 10 a.m. it was bled 6cc. The same precautions as to estimation etc., were taken as in last experiment. Further this experiment was a check on the last as the same corpuscles and corpuscular suspension were used in this case as in last. The M.H.C.D. dose was the same in all the estimation series, and the series were further the same in all their details, as regards grades of haemolysis in the various estimation tubes. Thus again bleeding a rabbit at one sitting to more than one third of the amount of blood in its body has no effect on the complement content of its serum. Douglas (1905-6) (44) gives the amount of blood in the buck rabbit as 4·85 % of the body weight. The amount of blood in this/

his case therefore is about 103cc).

These two experiments serve as a control by hewing that bleeding of itself has no effect on the omplement content of the blood for experiments to be iven later, where samples of blood are taken at in- ervals for the estimation of the complement content, nd also shew that very lavish bleeding has no effect n diminishing or increasing the complement content of he blood. Another interesting thing that appeared n both rabbits, was that the serum perfectly clear at irst became a day after the bleeding quite turbid nd remained so till the end of the experiment at east.*

REPETITION OF SACH'S EXPERIMENT.

The same precautions as regards corpuscles, sera etc. were taken here as in the last two experi- ments and it is unnecessary to repeat them.

January 11th. White doe, weight 2100 grammes bled 3cc. at 10 o'clock, serum separated, and 1cc of this serum added to 1cc 1% O.B.C. gave no haemolysis in 2 hours at 37°C. At 2 p.m. the leuco- cytes were counted, and they were found to be from counting two different dilutions twice, 10,600 per c.m. at 2·20 p.m. The rabbit was bled 6cc. at 2·20 p.m. There was injected into the ear vein 28cc of ox blood corpuscles washed thrice, equivalent to 56cc defibrinated ox blood at

3·25/

* This point is of great interest in connection with the preparation of sera for medico legal purposes - precipitin reaction. I intend to further investigate it.

3·25. The leucocytes count now, estimated as before, was 7,800 per c.m. At 4 o'clock, 4cc blood was drawn from the ear. Further 1cc blood was also drawn into citrate. The blood was washed. Heated immune body V.O.B.C. was added to the washed corpuscles and rabbit complement. The mixture was put into the incubator for 2 hours, haemolysis occurred shewing that ox blood corpuscles were circulating in the rabbits blood stream.

January 12th. The two samples of the rabbit's serum obtained yesterday were at 11 o'clock this morning estimated for their minimal haemolytic dose of complement. Two series were used for this estimation (1) with 1cc 1% suspension of O.B.C. + .015cc heated immune serum diluted 1 in 10 with saline + varying amounts of complement, with saline to make up to a uniform volume (2) in order to eliminate any error which might creep into the estimation, by the appearance of immune body in the rabbits own serum, a large excess of heated immune serum was added to this series. The series consisted of 1cc 5% O.B.C.+0·1cc. H.I.S. (whose M.H.D. for 1cc 1% suspension of O.B.C. fully complemented was ·002cc) varying amounts of complement with saline to make up to a uniform volume. I have here an example of the protocol of series I & 2

Estimation/

Estimation of M.H.D. of Complement obtained at 2.10 p.m. (before injection)

(I) With minimal haemolysic dose of immune body.

1% O.B.C.		H.I.S. diluted 1 in 10		COMPLEMENT before.		SALINE.	
1. 1cc	+	0.015c	+	.5	+	.0 = c	)
2. 1cc	+	0.015c	+	.4	+	.1 = c	) 2
3. 1cc	+	0.015c	+	.3	+	.2 = c	)hours
4. 1cc	+	0.015c	+	.2	+	.3 = c	) at
5. 1cc	+	0.015c	+	.1	+	.4 = ++++	) 37°
6. 1cc	+	0.015c	+	.05	+	.45= ++	) C.
7. 1cc	+	0.015c	+	.0	+	.5 = 0	)

(II) With excess of immune body.

5% O.B.C.		H.I.S. undiluted.		COMPLEMENT before.		SALINE.	
1. 1cc	+	0.1cc	+	.3	+	0 = ++++	)
2. 1cc	+	0.1	+	.2	+	.1 = +++	) 2
3. 1cc	+	0.1	+	.1	+	.2 = +++	)hours
4. 1cc	+	0.1	+	.0	+	.22 = ++	) at
5. 1cc	+	0.1	+	.03	+	.27 = ++	) 37°
6. 1cc	+	0.1	+	.01	+	.29 = 0	) C.
7. 1cc	+	0.1	+	.0	+	.3 = 0	)

Here/

Here with series 1. the M.H.D. before injection was ·2cc serum. With series 2 ·3cc gave marked & separate haemolysis while ·03 gave a trace and ·01 gave none, while with series 1.and series 2, after injection at 4 o.clock, the M.H.D.doses and the details of the series were exactly the same as before injection. At 1·40 p.m. the leucocytes per c.m.m. were 9400, at 2 p.m. the rabbit was bled 6cc, and 1cc was tested as before for ox blood corpuscles. The ox blood corpuscles were found to be present. No haemoglobin was present in the urine or the serum.

Wednesday, January 13th. The sample of serum obtained yesterday at 2 o'clock was tested this morning at 10·40, when series 1. and series 2. gave exactly the same results as yesterday. There was no Immune body present in the serum of this sample. At 1·30 the leucocyte count was 10,000 per c.m.m. At 2 o'clock 8cc were bled from the ear vein, and again 1cc blood was tested as before for ox blood corpuscles. The ox blood corpuscles were found to be present still in large quantities. There was no haemoglobin in the urine as yet.

Thursday, January 14th. The sample of serum obtained from the blood taken at 2 o'clock yesterday was tested for its complement content at 10·40/

10·40: when series 1. and series 2. gave exactly the same results as yesterday and the day previously. This serum when added to 1cc 1% O.B.C. and kept 2 hours at 37°C gave no haemolysis.

During the night, between January 13th. and 14th. the rabbit passed large quantities of haemoglobin in its urine and continued to pass it for the greater part of the day on January 14th.
At 11 o'clock the leucocyte count was 9000 per c.m.m. at 11·30 a.m. 8cc blood were obtained, and again 1cc blood was tested for ox blood corpuscles. This time no ox blood corpucles were found. At 3·10 p.m. the leucocyte count was 11,000 per c.m. 6cc blood was obtained at 3·40 p.m. The rabbit was still passing haemoglobin in urine but not so much as previously at 5 o'clock but between 5 p.m. and 9 p.m. the urine became absolutely free from haemoglobin.

Friday January 15th. The sera obtained from the blood taken yesterday at 11·30 a.m. and 3·40 p.m.was tested to-day at 10·30 for their complement content in both cases, series 1. and series 2. gave exactly the same results as they had given on every occasion previously. The serum obtained from the blood taken at 11·30 a.m. was much darker.

haemoglobin/

Haemoglobin tinted than was that obtained at 3·40 p.m. and while ·5cc of the 11·30 serum when added to 1cc 1% O.B.C. produced practically no haemolysis in 2 hours at 37°C ·5cc of the serum from 3·40 blood under the same circumstances produced complete haemolysis.

During the night the rabbit had passed large quantities of haemoglobin free urine. At 11·15 a.m. the leucocytes were 9,800 per c.m.m. At 12 o'clock the rabbit was bled 8cc.

Saturday, January 16th. At 10 o'clock the serum obtained from the blood drawn yesterday at 12 a.m. was tested for its complement content. Series 1. and series 2. gave exactly the same results in every detail that they had always done.

CONCLUSION.

This experiment confirms entirely Sach's experiment except in one thing: Sachs found a diminution of complement while there was absolutely no trace of such a diminution in this experiment. I do not pretend to explain it but I am confident of my result, for as explained above I did two series of estimations one with a MHD of IB, and one with a large excess of it, and in both series I got the same results. Another thing which this experiment brings out beautifully/

beautifully is the entire absence of any leucocytosis, which confirms what I had to say in a previous section So that this experiment shews that the immune body appears critically on about the third day and that even after the injection of 28cc ox blood corpuscles equivalent to 56cc ox blood there is not the slightest trace of dimunition of complement. This confirms and extends what I found previously (122) that the injection intravenously into normal rabbits of O.B.C. in sufficient quantity to use up the calculated amount of complement in the body of the rabbit, caused no diminution of that complement. I found further, in that research that the injection of sensitised or saturated (treated with 3 or 4 times the M.H.D. of Heated Immune serum) corpuscles intravenously into rabbits in an amount sufficient to use up the calculated amount of complement in the rabbit's body, again produced no diminution in complement. I now give an experiment in extension of this.

WILL THE INJECTION OF SENSITISED O.B.C. IN SUFFICIENT AMOUNT TO USE UP AT LEAST 4 TIMES THE CALCULATED AMOUNT OF COMPLEMENT IN A RABBIT'S BODY PRODUCE ANY DIMINUTION IN THE COMPLEMENT CONTENT?

Rabbit, normal buck, weight 2180 grammes. Taking the amount of blood in its body as 4·85% of the body/

body weight (Douglas) ( ) it amounts in this case to about 105cc, in which the serum or complement amounts to about 50% i.e. 52·5cc. The M.H.D. of this rabbit's complement for 1cc 1% O.B.C. with a minimal haemolytic dose of I.B. was 0·2cc, and therefore 2·5cc corpuscles is the calculated amount to use up all the complement in the rabbit's body. The rabbit was given 4 times this dose or 10cc washed ox blood corpuscles. These ox blood corpuscles were fully sensitised with 10cc Heated immune serum, whose M.H.D. for 1cc 1% O.B.C. was 0·1cc.

The rabbit was bled 8cc at 2·25. It was then injected at 2·30 with the 10cc O.B.C. + 10cc Heated Immune serum which had been in the incubator at 37° for 1 hour. The rabbit shewed no signs of distress and it was bled 8cc at 3 o'clock, 8cc at 4 o'clock and 8cc at 10·30. These samples were incubated for 1 hour and then stood at room temperature till next morning. Next morning the tubes were centrifuged and it was found that while there was no Haemoglobin in the serum before injection, there was a large amount ½ hour after injection, this was less 1½ hours after injection and much less though still present 8 hours after injection.

The M.H.D. of complement was estimated in all/

all 3, in series 1 and series 2. Series 1 had a minimal haemolytic dose of I.B. while series 2 had a large excess 0·15cc for 1cc 1% O.B.C. of a serum whose M.H.D. for the same amount of corpuscles was 0·01cc. This was done to exclude any mistake from some of the immune body injected with the corpuscles being still present in the serum and preventing comparison with the serum before injection. It was found that the complement after injection, ½ hour, 1½ hours and 8 hours, was in both series 1 and series 2, very slightly though definitely diminished.

I give here the Protocol.

Complement/

Complement undiluted: H.I.S. used had a M.H.D. for 1cc 10% O.B.C. = ·10cc of a dilution 1 in 10: 1% O.B.C. washed 6 times used.

SERIES I.

Before.

	1%O.B.C.		COMPLEMENT.		SALINE		H.I.S. 1 in 10.	½ hour after.	1½ hours after.	8 hours after.	
1.	1cc	+	·5	+	·0	+	·15 = c	++++	++++	++++	2 HOURS
2.	1cc	+	·4	+	·1	+	·15 = c	++++	++++	++++	
3.	1cc	+	·3	+	·2	+	·15 = c	++++	++++	++++	at
4.	1cc	+	·2	+	·3	+	·15 = c	+++	+++	+++	
5.	1cc	+	·1	+	·4	+	·15 = ++++	0	0	0	
6.	1cc	+	·05	+	·45	+	·15 = ++	0	0	0	37° C.
7.	1cc	+	·0	+	·5	+	·15 = 0	0	0	0	

Before.

	1% O.B.C.		COMPLEMENT.		SALINE.		H.I.S. undiluted.	½ hour after	1½ hours after	8 hours after.	
1.	1cc	+	·2	+	0	+	·15 = c	c	c	c	2 HOURS
2.	1cc	+	·15	+	·5	+	·15 = c	c	c	c	
3.	1cc	+	·1	+	1·6	+	·15 = c	++++	++++	++++	
4.	1cc	+	·08	+	1·2	+	·15 = c	+++	+++	+++	at
5.	1cc	+	·04	+	1·6	+	·15 = c	++	++	++	
6.	1cc	+	·02	+	1·8	+	·15 = ++++	0	0	0	
7.	1cc	+	·01	+	1·9	+	·15 = 0	0	0	0	37° C.

Incidentally these series shew a fivefold reduction of the M.H.D. of the complement in the second series by the addition of large excess of Immune Body. (See method of testing for complement

CONCLUSIONS.

DISCUSSION OF CAUSE OF SUDDEN DEATH IN IMMUNE ANIMALS ON INTRAVENOUS INJECTION OF CORRESPONDING ANTIGEN, DISCUSSION OF LEVADITI'S CRITICISM OF GRUBERS EXPERIMENT AND REPETITION OF GRUBER'S EXPERIMENT.

So far we have seen that the injection intravenously into normal rabbits whose serum is hardly at all or not at all lytic to ox blood corpucles, of large quantities of these corpuscles in one case 28cc. produces no diminution of complement: that the injection into normal rabbits of an amount of sensitised and saturated ox blood corpuscles calculated to be sufficient to use up all the complement in the rabbit's body. (McGowan (1908) (122)) produces no reduction of the complement, while under exactly the same circumstances 4 times the dose produces a distinct but hardly measurable reduction. Now Rehns, Schutze and Schiller and others have shewn that the injection of foreign corpuscles intravenously into an animal where serum is normally markedly lytic for these corpuscles causesmarked reduction of complement and in many cases the death of the animal, and I have shewn (loc.cit.) that the injection of comparatively/

comparatively small doses (2cc) of washed O.B.C. intravenously into rabbits which have been immunised against O.B.C., caused similar Phenomena. At first I thought that such phenomena were examples of "Anaphylaxis". (If an animal say a guinea pig is given a small dose of albumen say horse serum subcutaneously and a fortnight after given say 5cc of horse serum subcutaneously, it dies in many cases almost immediately after the injection. This is a particular instance of "Anaphylaxis" or hypersensitiveness.) But now after having done experiments to try to settle this question I believe they are not so. As regards the reduction of complement I believe it is explicable by failure of time for regeneration and by absorption by the lysed stromata of the red corpuscles. This reduction of complement happened to the greatest degree in rabbits whose serum was very highly lytic, thus determining a rapid solution and formation of stromata and again in rabbits which died rapidly after an injection - say five minutes, thus preventing a regeneration of complement. Again the deaths to my mind were not due to "anaphylactic" phenomena, for they took place with greater frequency and more suddenly the greater the strength of the serum of the animal in I.B. (Immune Body). Death did not take place on subcutaneous or intraperitoneal injection, but always on intravenous. An immune animal/

animal might be very seriously ill to-day after an injection intravenously and to-morrow on a further intravenous injection be so ill as to die, which is quite different from anaphylactic phenomena. (Under typical anaphylaxis, there would have been an immunity to the second injection). Two animals after having received the same number of injections subcutaneously at some intervals might be tested on the same day intravenously with the same dose and one might die and the other show absolutely no symptoms. Again feeding on blood and a minute injection of blood do not sensitise, (Unlike what happens in anaphylaxis.). And further all the symptoms produced by injection in these immune animals were produced in a normal animal into which I injected intravenously 4cc O.B.C. + 4cc H.I.S. which mixture contained little flocculi of agglutinated ox corpuscles. These symptoms are great dyspnoea and collapse. Coca (36) attributes the symptoms to blocking of the lung capillaries and with this view my observations lead me to agree. Levaditi ( ) has further shewn in guinea pigs immunised against pigeon corpuscles that an intravenous injection of these corpuscles causes death by blocking up the capillaries of the lung in which case the pigeon corpuscles being easily recognised are seen microscopically in sections.

Here I should like to refer more fully to some statements of Levaditi (112) with regard to what happens/

happens in fresh guinea pigs whose serum is normally lytic to pigeons corpuscles on the intravenous injection of these corpuscles. He comes to the conclusion that the haemoglobinuria is due to phagocytosis in the spleen and elsewhere breaking up the corpuscles and that there is no extracellular breaking up. He finds that the pigeons corpuscles persist unchanged in the blood of the guinea pig for at least 1 hour 40 minutes. There is never at any time, he states, any extracellular solution of them. He finds that in less than 40 minutes the macrophages of the spleen are filled with the hen corpuscles and the latter are undergoing intracellular digestion. He concludes that in the fresh guinea pigs the absence of extracellular haemolysis and the intense erythrophagocytosis which takes place in the spleen explains why these guinea pigs have no symptoms on injection of pigeon corpuscles and by implication explains thus the haemoglobinuria and the breaking up of the pigeon corpuscles. Now I have injected rabbits - choosing individuals whose serum was markedly lytic to hens corpuscles - intravenously with 2cc washed hens corpuscles and I have obtained specimens of the organs spleen, liver, kidney, and lung, at 10, 20, 30, and 60 minutes after injection. I found that haemoglobin appeared in/

in the urine in less than 20 minutes after injection and on looking at my specimens of spleen etc. instead of finding in the spleen every field of the microscope a heaving mass of engorged phagocytes as one would expect from his statements, I found at the end of an hour after injection a few instances of phagocytosis by Kupfer's cells in the liver and one instance of phagocytosis by a liver cell, but never a single instance of phagocytosis in the spleen. In fact it is extremely difficult to find hen corpuscles at all in the spleen at the end of half an hour. After this it is a detail to say that the haemoglobin appears in the urine far too quickly after injection to be caused by phagocytosis. Levaditi, in his criticism of Gruber's experiment, states that the lyses does not take place in the circulating blood, that the Heated immune serum is absorbed from the peritoneal cavity, sensitises the guinea pigs corpuscles which corpuscles are then actively phagocyted by the macrophages of the spleen. For the present I judge of the validity of this statement from what Levaditi stated to be the case when guinea pigs were injected intravenously with pigeon corpuscles and what I actually found to be the case in the exactly comparable experiment where rabbits were injected with hen corpuscles. I shall now proceed to give the details/

details of a repetition of Gruber's experiment.

REPETITION OF GRUBER'S EXPERIMENT.

EXPERIMENT. Two guinea pigs were inoculated intraperitoneally with 2cc rabbit blood corpuscles, on January 4th, January 8th, January 14th. Both guinea pigs were bled to death on January 18th, and the serum separated from the blood clot. The serum was very actively lytic for rabbits red blood corpuscles. It was now inactivated by heating to 56°C. for ½ an hour. Now a rabbit was taken on January 18th. and its red blood corpuscles counted at 10·40 and found to be 6,950,000 per cmm. The weight of the rabbit was 2370 grammes. Then 7cc of the inactivated guinea pig immune serum was injected intraperitoneally into the rabbit at 12·30. At 3.40 the R.B.C. count was 7,500,000, per cmm. No Haemoglobin in urine. At 7·30 p.m. and 9.30 p.m. there was no Haemoglobin in the urine. January 19th. 9 a.m. no Haemoglobin in urine. At 3 p.m. there was Haemoglobin in urine and in the serum. Now blood was drawn into a paraffined pipette from the ear vein and this blood was centrifuged, the whole operation being done in 4 minutes and the supernatant plasma was found to be markedly haemoglobin/

haemoglobin tinted. Again the rabbit was bled from the ear vein into an ordinary centrifuge tube and the blood centrifuged in a hand centrifuge. The whole operation bleeding and centrifugation was complete in 3 minutes and a dark haemoglobin tinted plasma was obtained which clotted in less than a minute after removal from the centrifuge. Again the rabbit's ear was covered with liquid paraffin, blood was drawn into a centrifuge tube lined with paraffin, and this blood was centrifuged, the whole operation occupying 2 minutes by the watch. The plasma above was deeply haemoglobin tinted and coagulated in about 2 minutes after removal from the centrifuge.

January 20th. 9·30 Haemoglobin still in urine and serum: R.B.C. count = 1,600,000 per cmm. A microscopical film of the blood, at this stage, shewed a very large number of shadow, swollen corpuscles with no haemoglobin. At the same there was a large number of microcytes and extreme poikilocytosis. There was no phagocytosis seen in the leucocytes of the blood. Now Levaditi has stated that there is absolutely no lysis taking place in such conditions extracellularly, and that phagocytosis is the explanation of the whole phenomena. How comes it then that there are such things as free shadow cells, poikilocytes and microcytes/

microcytes? Levaditi has further brought forward the objection that the haemoglobin as seen in the serum of such an animal appears in the serum at the instant of coagulation which coagulation sets free complement which at once lyses the sensitised corpuscles. I have shewn above that if such lysis takes place it must do so inside 2 minutes, and now I record an experiment which to me absolutely confutes Levaditi's objection. I took a centrifuge tube, placed in it ½cc of a 50% suspension of ox blood corpuscles suspended in a specific heated immune serum whose M.H.D. for 1cc 1% suspension of O.B.C. was ·01cc. I kept this suspension in the incubator for 1 hour: I then drew 1cc blood from a fresh rabbit into this suspension: centrifuged it leisurely, and found that there was absolutely no Haemoglobin in the supernatant fluid although it was quite coagulated. The time taken to perform the experiment was 10 minutes, a much larger time than taken in any of the experiments recorded above. In this experiment on Levaditi's hypothesis every condition was the same as in drawing a sample of blood from the ear of an animal in which Gruber's experiment was being performed. The drawing of the blood from the normal rabbit into the tube of sensitised corpuscles ought on Levaditi's hypothesis to have caused haemolysis owing to coagulation, phagolysis and setting free of complement. That it did not cause/

cause haemolysis in even a much longer time than Levaditi gives only goes to shew that Levaditi's hypothesis is wrong and that from this and other facts noted above complement is not formed from the leucocytes on coagulation, that it pre-exists free in the blood, and that the haemolysis in Gruber's experiment takes place in vivo: and there extracellularly.

WITH A VIEW TO OBTAINING SOME KNOWLEDGE OF THE SEAT ORGAN OF COMPLEMENT WE WILL NOW CONSIDER WHAT EFFECT THE EXTIRPATION OF THE SPLEEN HAS ON THE COMPLEMENT CONTENT OF A RABBIT'S BLOOD.

March 4th. Rabbit, buck, bled 7cc at 2 p.m. Spleen removed at 3·30 bled again 6cc at 7 p.m. March 5th. 10 o'clock yesterday's samples of blood estimated for their complement content: 2 p.m. Rabbit again bled 5cc. March 6th. 10 o'clock yesterday's sample estimated for its complement content: 2 p.m. 5cc blood again taken. March 7th. yesterday's sample again estimated for its complement content. The same corpuscles were used in all the estimations, and the minimal haemolytic dose of the complement was the same in them all

On/

On March 17th. the complement was again estimated this time with new corpuscles. The minimal haemolytic dose was very slightly greater, but as in a control rabbit (see below) done at the same time the M.H.D. was also slightly greater the change may be put down to the corpuscles and not to a diminution of complement. So that Splenectomy had no effect on the complement content of the blood. During the two days following it and 13 days after, any effect, instead of being of the most marked character, is hardly demonstrable and can be attributed with justice to the new corpuscles used in its estimation.

HAS THE REMOVAL OF THE THYROID ANY EFFECT ON THE COMPLEMENT CONTENT OF THE BLOOD?

Rabbit bled 6cc at 1·30 p.m. March 8th. Thyroid removed at 3 p.m. Rabbit bled again 6cc at 7 p.m. March 9th. 10 a.m. complement estimated in the two samples blood obtained yesterday. Bled 5cc at 2 o'clock: March 10th. complement of yesterday estimated. Bled 5cc at 2 o'clock. March 11th. Complement of yesterday estimated: bled 6cc at 2 o'clock: March 12th. complement of yesterday estimated: same ox blood corpuscles used for all the estimations. M.H.D. of complement was the same in all the cases: March 17th. complement/

complement estimated with new set of corpuscles and as above practically no change found in it. This rabbits complement content acted as a control to the one above. Such a control points to the very slight diminution in both being due to a change in the resistance of the O.B.C. or in the concentration of their suspension. Therefore the removal of the thyroid had no effect on the complement content of the blood certainly within 3 days and most probably within 9 days.

I conclude therefore from the experiments in this section that the complement exists as such in the circulating blood and that it is not first formed by phagolysis from the leucocytes on coagulation. Where is it formed then? One thing that one particularly notices in working with complement is that there seems to be a certain complement tension so to speak for each animal, above or below which the complement cannot be made to vary, even by very drastic treatment of the animal e.g. extensive bleeding. (See below) That the extirpation of the spleen and of the thyroid should have no effect on the complement content of the blood may be taken I think as presumptive evidence that they are not the seat of its formation. That/

That immunisation with living bacteria, which without doubt produce a leucocytosis, causes no increase in complement, may be taken as some evidence however slight for the view that the bone marrow does not form it: and Busse's experiments with cinnamic acid leucocytosis confirm this. When on the other hand we consider the tremendous strains that may be put on the seat of formation of complement wherever it is and to which it is equal, e.g. by removing half of the blood of the body in a short time and again by the injection of sensitised corpuscles in more than sufficient quantity to use up the calculated amount of complement in the rabbit's body and these without effect on the complement content of the rabbits blood, we may conclude I think that the organ which produces the complement is one of great metabolic activity. - The liver is pre-eminently such an organ. Were the suprarenals and the endothelium of the blood vessels excluded one could say with some justice that the exclusive evidence pointed definitely to the liver as the seat of formation of complement. As it is one can only say that the balance of exclusive evidence points in this direction and even this is only warranted by the fact that not the slightest presumptive evidence has been brought forward for either the suprarenals or the endothelia having anything to do with the formation of complement.

SECTION III

CONSIDERATION OF THE POSSIBILITY OF THE LIVER BEING THE SEAT OF FORMATION OF THE IMMUNE BODY AND COMPLEMENT.

GENERAL CONSIDERATIONS & LITERATURE.

If the circulation in an animal is suddenly stopped without loss of blood, say by pithing and the blood estimated in the various organs it is found that under these conditions the liver contains a quarter of the blood in the body. But this is not the maximum of blood that the liver can contain for if the liver be excised, and the hepatic veins ligatured, it will be found that a quantity amounting to more than ½ of the blood in the body can be injected by the portal vein into the liver without any rupture of the vessels. I have performed this experiment on several occasions. These facts shew, and the histological appearances confirm it, that the liver is like a sponge, and the amount of blood it contains, though always large, can undergo considerable variations.

Further, the low pressure in the portal vessels, the narrow capillaries, the angles and corners and the large amount of blood in the liver favour/

favour the catching up of foreign particles from the blood stream and their retention for a long period in the substance of the liver. Ponfick (1869)(156) and others have found that foreign particles, such as Cinnabar, Chinese ink, carmine etc. after intravenous injection, tend to accumulate more in the liver than in any other organ. As to what happens to these particles in the liver, an idea will best be gained by a study of the paper of Herring and Simpson(87) "On the relation of the liver cells to the blood vessels and lymphatics." In that paper, they quote a large amount of literature, including the work of Schafer, shewing the presence of intracellular canaliculi in the liver cells, which can be injected only from the blood vessels. They, themselves, found these intracellular canaliculi in the liver cells of the rat, frog, rabbit, guinea pig, hedgehog, dog, cat, ferret, monkey, fowl and pigeon. They found further that the cytoplasm of the Kupfer's cells is usually injected by the method used for demonstrating the intracellular canals mentioned above but think that this is due to vital absorption of the injection material. They also quote a specimen of Mr.Richard Muir's from a chloroform poisoning case which shews what looks like injection, by fat, of these canaliculi. They/

They themselves have also found haemoglobin crystals inside the nuclei of the liver cells. They quote Browicz as having found the same thing, and take this as evidence of the Red blood corpuscles passing into the liver cells and being broken down there. Herring and Simpson, further, give a picture in this paper of a red blood corpuscle inside a liver cell; and they conclude from this, that there must be an intimate relation between the blood in the intralobular vessels and the liver cells. Further they conclude that, in the liver of the dog and cat, the lymphatics are confined to the visible connective tissue of Glisson's capsule and the adventitia of the hepatic veins. There are no lymphatics within the lobule. The perivascular lymphatics described by McGillavry do not exist. They quote Kupfer, as having shown in 1898 that the cells, named after him, are endothelial cells of the blood vessels and not connective tissue cells, lying outside the blood vessels. Browicz independently came to the same conclusion as Kupfer. In 1902 Schäfer ( ) (167) shewed that the liver cells can be injected from the portal vein, and that there is a direct communication by means of fine channels between the lobular capillaries and the interior of the liver cells. He could find no trace of perivascular lymphatics within the lobules. To quote further/

further from their paper, in 1869, Ponfick shewed that after fine particles of cinnabar have been injected intravenously into rabbits, guinea pigs, and dogs, some hours before death, a post mortem examination of the liver shows that many of the granules of cinnabar lie in round or oval amoeboid cells which may be mistaken for liver cells. In 1876 Kupfer described his cell and noted its phagocytic power. Asch found that Kupfer's cells rapidly take up particles of cinnabar and carmine when these are injected into the blood. Lowit concludes that Kupfer's cells take up red blood corpuscles and transfer their haemoglobin to the liver cells. The phagocytic properties of these cells have been amply shewn by Kutimeyer, Siebel and Heinz. Kupfer stated in 1898 that the Kupfer cells are an integral portion of the capillary wall. He found, further, 12 hours after injection of defibrinated rabbit blood intravenously into the rabbit, red blood corpuscles in these cells. In his paper he gives a drawing of such a Kupfer cell crammed with Red blood corpuscles. He thinks that the endothelium has the form of a syncytium and that no limits can be demonstrated between the cells. Browicz however does not think that they form a syncytium. Herring and Simpson conclude that the endothelium, which lines the intralobular blood spaces, (sinusoids in the sense of Minot) is incomplete and allows the passage through it both of fluid and of fine/

fine solid particles into the liver cells. The endothelial cells are of two kinds, large and small. The large cells, Kupfer's cells, are phagocytic and often project into the blood spaces. It will be seen further, that in the above some evidence is brought forward for the liver being the seat of breaking down of the animals own effete blood corpuscles. (Herring and Simpson pge. (496.) ; Kupfer pge.(—))Haemoglobin crystals have been found inside the nuclei and Red blood corpuscles inside the liver cell protoplasm: Paton, Goodall and Gulland (1903-05) (76) have shewn that the spleen is not the seat of breaking down of the red blood corpuscles. Taking these things into consideration, together with the mechanics of the circulation in the liver and the nature of the endothelium of the intralobular capillaries, the fact, small in itself, that the bile, being the waste product of the red blood cells actually comes from the liver, it is very probable, as some believe that the liver is the organ which rids the body of one fairly foreign particulate body from the blood stream - effete blood corpuscles - in other words the liver is very probably the seat of destruction of effete blood corpuscles: but no haemolysin is formed against its own corpuscles, or if it is formed there is always an antihaemolysin formed to keep it in check. This is as/

as might be expected, for an autohaemolysin uncontrolled would be a distinctly dangerous thing in the animal economy. There is thus some evidence for the physiological process of breaking down of the animals own effete blood corpuscles taking place in the liver. There is also at the same time a large amount of evidence for the possibility of the liver being the seat of breaking down of, and of initiating stages for the formation of antibody against, other particulate material of a much more foreign nature.

Weber (1909) (196) in a case dead of pneumonia with jaundice, found a great amount of dividing phenomena in the nuclei of the liver cells, and in all the cells, possessing such a hypertrophied nucleus, he found not only in the body of the cell, but even inside the nucleus, the pneumococcus. In volume 11. of the English edition of Van Noorden's Pathology of metabolism, there are numerous references, shewing that the liver can modify the action of certain pharmacological substances such as alkaloids, as well as certain substances, more definitely known as toxins. The method of experimenting used for such determinations was that of ascertaining the minimal lethal dose when injection was performed by the ear vein and by a mesenteric vein. It was found that the dose by the mesenteric vein was much larger/

larger than by the ear vein. This of course may be due to the liver destroying the poison, say in the case of strychnine, before it gets to the highly specialised cells of the anterior horn of the spinal cord: but such rapid destruction is not absolutely necessary for protection, for the dilution, stagnation, and retention in the liver would sufficiently account for the observed results. By the same method, some find that the liver has a neutralising action on the poisons prepared in the intestine; others find that it has no such action. Petrone and Pagano (1907) (149) sum up their own and some other Italian work on this subject. They state that, without doubt, the liver has a protective action against the vegetable alkaloids. The method of action may be the formation of stable compounds with the albumens of the liver cell, which compounds are afterwards transformed into innocuous substances by the ultimate metabolism of the cell, or eliminated by the bile, or turned out afterwards slowly into the circulation in such a manner as not to affect the organism. Lastly the protective action of the liver may be due to the great dilution of the poison in the large amount of blood in the organ. It possesses, too, according/

according to P. and P. a protective action against several mineral poisons, and against substances gaining entrance by the intestines, such as ammonia, the aromatic substances, the peptones, albumen, soaps and alcohol. It also protects against foreign sera and against blood coagulating substances. Several authors also state that it protects against carbamic acid derived from intestinal absorption, as animals with Ecks fistula shew all the symptoms of carbamic acid poisoning. The liver, too, according to P. and P. protects against such substances as bicarbonate, sulphate, and chloride of soda, adrenaline, ether, chromic acid, and phosphorus. It,too, possesses a protective action against microorganisms,such as anthrax, tubercle, staphylococcus aureus, and oidium albicans. It has no protective action against streptococcus; and its action on B. Coli is uncertain, some finding injection by the mesenteric vein to be less lethal as compared with injection by the ear vein, while others find no such difference on injection by the mesenteric vein. They state further that the liver exercises a protective action against tetanus toxin, cholera toxin, toxin of dysentery bacillus, certain B coli products, alcoholic extracts of putrescent, and of typhoid materials, against alcohol soluble/

soluble products of B. pyocyaneus , and against diphtheria toxin. With regard to some of these, the results obtained are conflicting. As regards the mechanism of the protective action against bacteria, according to some, it is due to the phagocytic activity of the endothelium of the liver capillaries. Some, however, think that it is related to the glycogen function of the liver. P. and P. themselves in this research used the method of injecting by ear vein and mesenteric vein and the substance injected was the aqueous extract of infants fæces sterilised by fractionally heating to 58°C for several days. They considered the general condition, the temperature and the blood in the animals injected. In all these respects, in every case, those injected by the mesenteric vein had the best of it. They conclude that the liver exercises an undoubted protective action against the intestinal poisons. With regard to the experiments of P. and P., and other experiments of a similar nature, one has to be very guarded in drawing any conclusions from them, as to the liver being the seat of formation of antibodies. Were the results of the experiments absolutely trustworthy, and the amount of conflicting results renders this doubtful, the utmost conclusion that one could draw would be that the liver by dilution/

dilution of the poison, or fixing of it, prevents it getting at the highly specialised cells and so causing the death of the animal. It shews that the liver tends to filter out toxic bodies, both fluid and particulate, from the blood stream, and to keep them inside itself, and so prevent them doing harm. After this, there is, however, more chance than in any other organ, for the antibodies being formed, owing to the large amount of antigen present in the organ and owing to the intimate relationship possible with the liver cells. Delzenne (1898) (40) shewed that the liver was the seat of formation of antithrombin.

Ehrlich (1900) (54) found that if he poisoned a rabbit with a dose of phosphorus, which killed it in 3 days, and took the serum on the second day, the serum had lost its former solvent power for guinea pigs corpuscles. It was reactivated by adding a large amount of guinea pig serum, shewing that in Phosphorus poisoning, with affection of the liver, the complement is diminished. Von Bergmann and E.Savini (1907) (12) found in phosphorus poisoning a disappearance or diminution of the complement. Nolf (1908) (141) by cutting the liver out of the circulation in rabbits, found the complement diminished after the operation and concludes that the liver is the seat of formation of the complement. To shew further the possibilities of the liver, Jacoby, Doyon, Nolf, Morawitz find in phosphorus/

phosphorus poisoning, together with the necrosis by the liver, an incoagulability of the blood and a disappearance of fibrinogen; and Doyon and Policard (45) have observed the same phenomena in chloroform poisoning.

SUMMARY OF LITERATURE.

To sum up then, evidence had been brought forward from the literature, that material, injected into the circulation, tends to accumulate and be retained in the liver, where, the close association with the liver cells permits at least the possibility, more than in any other organ, of changes being effected. Further some more direct evidence has been adduced for the liver being the seat of formation of complement.

ATTEMPT TO SHEW LIVER SEAT OF FORMATION OF IMMUNE BODY BY EXTRACTION METHOD.

Before I appreciated the power of organ extracts of themselves to absorb complement, I did several experiments by this method, to see if the liver might possibly be the seat of formation of Immune body. I give them here, for if they serve no other purpose, they shew the impossibility of arriving at any results by this/

this method.

EXPERIMENT I. and II.

Two rabbits were taken, and their serum was ascertained to be free from normal lysin for ox blood corpuscles. They were then injected with 2cc. ox blood corpuscles intravenously and killed 24 hours after injection by bleeding: by this time no immune body was present in the serum. Was it present in any organ extracts? The liver, the spleen, kidney and suprarenals were chopped up and extracted with saline and with the rabbits own serum. These extracts were tested then for immune body, the saline one by adding it together with fresh complement to ox blood corpuscles suspension and incubating; the serum one by adding it alone to ox blood corpuscles suspension and incubating. No evidence of I.B. was forthcoming: and none could be expected for the tissue detritus absorbed the complement.

EXPERIMENT 3.

A rabbit without normal lysin in its blood was injected intravenously with O.B.C. Three days after injection it was bled to death. Its serum which contained I.B. was used to extract the organs of a fresh control rabbit, and the serum of a fresh control rabbit was used to extract its organs. Neither of the extracts, however,/

however, shewed any signs of I.B. although without doubt it was present in the serum of the animal which had received the O.B.C. injections.

EXPERIMENTS 4 and 5.

2 Rabbits, without normal lysin in their blood, were injected intravenously with O.B.C. Two days after this injection, they were injected, one intravenously, and one subcutaneously with O.B.C., to try to stimulate, by the presence of these foreign cells, the production of the I.B. Three hours after this injection, they were killed and their serum shewed no I.B. whilst the extracts of the various organs again shewed no I.B.

EXPERIMENTS 6 and 7.

Two rabbits taken: they had been injected frequently with O.B.C.; and the M.H.D. of I.B. in their serum for 1cc. 1% O.B.C. was about ·00-
-2cc. These rabbits were killed by bleeding and their organs extracted with their own sera, to see if any of the organ extracts contained more I.B. than the corresponding serum. The M.H.D. of the serum itself and of the extracts made with that serum was the same as regards immune body content, shewing that there was no accession from any of the organs, and further, that the organ extracts do not absorb immune body: while on the other hand/

hand even 1cc. of the serum extract, owing to the absorption of complement, gave no trace of haemolysis with a suspension of O.B.C.

ATTEMPT TO SHEW LIVER THE SEAT OF FORMATION OF IMMUNE BODY BY MEANS OF PERFUSION EXPERIMENTS.

Next I tried to solve the question by means of perfusion experiments.

EXPERIMENT I. and II.

Rabbit: no normal lysin in its serum, injected intravenously with O.B.C: killed after 2 days by bleeding: no I.B. in its blood: the portal vein and inferior vena cava below the liver were ligatured and a cannula was placed on the inferior vena cava above the liver. The liver was kept in normal saline at 37°C: it was then injected by means of the cannula with 40cc. of 1% O.B.C. in the rabbits own serum. After 2 hours at 37°C some of the fluid was withdrawn and found to be markedly haemoglobin tinted: but in a control rabbit done at the same time the same result was got. Some of the injecting fluid, kept in a glass vessel at 37°C, shewed no haemolysis after 2 hours: the haemolysis, got in the experiment as well as in the control, was most likely/

likely, due to either the bile from ruptured bile capillaries or to autolytic products of the liver. This experiment was repeated with the same result.

EXPERIMENT III and IV.

Rabbit, no normal lysin in its serum. Injected intravenously with 2cc O.B.C.; killed by bleeding 2 days after: no Immune body in its blood. Liver taken, kept in a saline bath at 37°C and perfused with 1% O.B.C. in saline. The corpuscular suspension was kept circulating by means of a Higginson's syringe at a low pressure controlled by a mercury manometer. Samples were drawn off at various intervals: complement was added to these and they were incubated but there was no haemolysis, shewing that I.B. had not been given off from the liver cells to the circulating corpuscles. This experiment was repeated on another rabbit with the same result.

FURTHER EXPERIMENT TO THROW LIGHT IF POSSIBLE ON THE SEAT OF FORMATION OF IMMUNE BODY.

Next I tried the following experiments without being able to get any evidence to incriminate the liver.

EXPERIMENTS I. and II.

A rabbit, which had been immunised against ox/

ox blood corpuscles and whose serum was very actively lytic to O.B.C., was injected, intravenously, with 4cc O.B.C. Half an hour after injection, it was killed by pithing, the abdomen was immediately opened, and the liver ligatured out of the circulation. Blood was then obtained from the liver and from the general circulation and the M.H.D. of I.B., in each, ascertained. It was found to be the same in each. This experiment was repeated with the same result. So that these experiments as far as incriminating the liver goes, again failed.

LITERATRUE DEALING WITH THE INJECTION OF NUCLEATED CORPUSCLES INTRAVENOUSLY INTO MAMMALS.

Levaditi (1905) (172) injected fresh guinea pigs intravenously with washed pigeon corpuscles, in the amount of 1 to 1·2cc of a thick emulsion. According to Levaditi, 0·5cc fresh guinea pig serum dissolved, completely, a drop of pigeon blood. Taken roughly, the drop is equivalent to about 1cc of a 5% suspension of the corpuscles. He found that, if one examined the blood of fresh guinea pigs 5 minutes, 30 minutes, 40 minutes to 100 minutes after the injection of pigeon blood, one found that they persisted, unaltered, in the blood up to more than an hour. They agglutinated round masses of platelets and put themselves in intimate contact with the polynuclear leucocytes. At no time could one see, in the blood, any/

any evidence of extracellular solution of these nucleated erythrocytes. Further, if one killed the animal, at a time when the pigeon corpuscles had disappeared from the general circulation, and concentratedattention on the spleen, one saw that very rapidly, sometimes in less than 40 minutes, the macrophages of the organ are filled with nucleated corpuscles and that this erythrophagocytosis is extremely pronounced. It is easy to seehe says that phagocyted corpuscles are much more numerous than the free corpuscles, and that the englobed corpuscles lose no time in shewing marked signs of intraprotoplasmic digestion. Of all the other organs, the liver is the only one which shews R.B.C. phagocyted by the Kupfer's cells. On the contrary neither the lymphatic glands nor the lungs contain the least trace of these corpuscles. He concludes, then, that the haemolysis of the pigeon corpuscles in fresh guinea pigs is due to phagocytosis entirely.

EXPERIMENTS OF INJECTING WASHED HENS CORPUSCLES INTRAVENOUSLY INTO RABBITS.

Two years ago at the commencement of this work, I did analogous experiments, by injecting Rabbits intravenously/

intravenously with washed hens corpuscles. Many rabbits normally have a serum lytic for hens corpuscles - in some of the rabbits used this was such, that 1·5cc rabbit serum completely lysed 1cc 5% suspension of hens corpuscles in 2 hours at 37°C. Some rabbits, on the other hand, have a serum hardly or not at all lytic to hens corpuscles, 1·5cc producing practically no lysis in 1cc 5% hens corpuscles in 2 hours at 37°C. Both sets of rabbits, however, produce increasing quantities of lysin for hens corpuscles on injecting them with hens corpuscles.

9 rabbits, with lytic serum, were taken and they were each injected intravenously with 2cc hen corpuscles + 2cc saline. They were killed, at the following intervals after injection. 10, 20, 30, 60, 90, 120, 300 minutes and 24 hours and 48 hours. Haemoglobin was present in the urine in 20 minutes and disappeared from the urine in 5 hours. No hens blood corpuscles or rabbits blood corpuscles were found in the urine. Specimens from the liver, kidney, spleen, and lung were fixed in alcohol, saturated Hg. Cℓ2 in normal saline, and in 10% Formalin immediately on the animal being killed. Sections were eventually made from specimens from all three fixatives and stained with haematoxylin and eosin. On examination of these sections,

hen/

hen corpuscles were never seen in the lung or kidney; they had, absolutely, disappeared from the spleen in half an hour; and the only organ, in which they could be found, was the liver in which they were present in large numbers. They could be found in the liver up to 5 hours. Phagocytosis was never observed in the spleen; to occur, it would have had to occur very suddenly before 30 minutes, as the corpuscles had disappeared from the spleen in 30 minutes. The only place, that phagocytosis was observed in was in the liver, and here, by Kupfers cells and by liver cells. An example of phagocytosis by a liver cell is given (plate 1, figure 4); and a good number of other examples of hen corpuscles inside liver cells were seen. Phagocytosis by Kupfers cells was fairly frequently found. The phagocytosis, however, was scanty and in no way could it explain a very small fraction of the haemolysis; and the haemoglobin appeared in the urine before phagocytosis was detected.

In another series of experiments, three rabbits were taken, with sera, hardly lytic at all to hens corpuscles. They were injected intravenously with 2cc. washed hen corpuscles and 2cc. saline. They were killed at the following intervals, 1 hour,

5/

5 hours and 24 hours after injection. There was no haemoglobin in the urine of the rabbit killed after 1 hour, thus corroborating, what had been found with its serum in vitro. Specimens from the kidney, liver, lung and spleen, of all three, were fixed in alcohol, saturated $HgCl_2$ in saline, and 10% Formalin of these sections were afterwards cut and stained. The results obtained here were that, from examination of films of blood drawn at various intervals from the rabbits ear vein after injection, it was found that the hen corpuscles had disappeared from the films, (and so from the general circulation,) in 20 minutes. In the rabbit killed 1 hour after injection, no hen corpuscles were to be seen in the kidneys or lungs: and while very few - one in every 3 fields of the high power - were to be found in the spleen, (figure 5 , plate 1) gives some idea of the state of matters in the liver. The liver capillaries are actually crammed with them: and this illustrates well, the sifting out power of the liver. The corpuscles are present in such numbers here, where the rabbits serum was lytic the corpuscles to a large extent had been dissolved and so there were fewer to give rise to such an appearance in the liver because of the non-lytic nature of the serum. Very little phagocytosis was seen in the liver and none at all in the spleen or other organs.

It will be seen, therefore, that on intravenous injection of hen corpuscles, they tend to be sifted out and to accumulate in the liver, being present/

present there, in very large numbers, when there are very few or none at all in the spleen: also, that phagocytosis was never present in such quantity to account for even a small fraction of the haemolysis and haemoglobinuria, and the only organ in which phagocytosis was observed was the liver, and there the phagocytosis took place in Kupfer and liver cells. The accumulations in the liver and the phagocytosis there in no way explain the extremely rapid haemolysis and haemoglobinuria. The haemolysis takes place in the general blood stream and extracellularly. Gruber's experiment and this experiment shew this. The accumulation in the liver is an expression of the mechanics of the circulation, while the phagocytosis, I take it, is the beginning of the process that ends in the formation of the specific antibody: antibody formation, whether to foreign red blood corpuscles, nucleated or non-nucleated, or to bacterial bodies may therefore be of the same nature as the destruction of the animals own effete blood corpuscles: That the immune body should circulate in the blood, in the one case and not in the other, may be another way of expressing the foreign nature of the one set of bodies and the more familiar nature of the other. In addition to what has been said previously about the structural and functional advantages the liver has over other bodily organs for the formation of immune body/

body, I would mention, here, some other facts. In the first place a great amount of change can take place in a liver cell, during glycogenic functioning in the rabbit and yet that cell recover. ( ) Plate (I) shew 3 stages (1) with no glycogen, (2) with small amount, and (3) with large amount. The necrotic badly fixed and degenerated look of the liver cells can be much more advanced even than in figure (3.) and yet the cell recover. This must indicate great anabolic and Katabolic swings in the metabolism of the liver cell. Again as in figure (1) plate 2 the liver cells are in a great many cases in the rabbit found to be crowded with large vacuoles. Whether these vacuoles are of a phagocytic or storage nature I do not know, but having regard to the possible functions of the liver cell, and the peculiar shape of the vacuoles (indenting the nucleus) I would consider them to be phagocytic. This view is strengthened by a consideration of two cases of Dr.Stuart MacDonald's to which he has kindly allowed me to refer. The first case is that of a primipara who was ill the day before labour came on and who died a few days after. The liver cells in this case at the periphery of the lobules are highly vacuolated and, in some of these vacuoles, blood corpuscles are found, Figure (1 & 2) plate (iii) are from this case. The second case is one of lympho sarcoma invading the liver and here the liver cells are apparently phagocyting the lymphoid cells.

SECTION/

SECTION IV.

I have shewn that there is no evidence for the usually accepted organs, the spleen, bone marrow, and lymphatic glands, being the seat of formation of immune body and complement. The mind should therefore be kept open for the possibility of some other organ being found to be the seat. I have brought forward evidence in this paper exclusive and circumstantial for the possibility of the liver having this function. When we consider that antibodies can be produced to antigens by feeding animals on these antigens, it would appear that the formation of these antibodies may be an example of the way in which the body physiologically deals with the overflow from the intestine of liquid and particulate albumens which have not undergone complete intestinal digestion and are in consequence material foreign to the organism. The production of antibodies, therefore, may be taken to be a physiological function of the body which gets exercise under so called normal conditions by the overflow of undigested material from the intestine.

It is only in exceptional cases, such as those where the factors are of the nature of those in a pharmacological experiment as regards accuracy of dose &c. that such antibody formation of itself has been/

been shewn to account for the acquired immunity of an animal. This is the case for instance, in the production of antibodies to the toxins of diphtheria and tetanus. If however we consider bacterial infections, many more and much more complex and less accurate factors come into play than in a pharmacological experiment or in the production of antitoxin. There are, in addition to those which are common to the toxin conditions, the multiplication and morphological defensive changes (such as encapsulation as in streptococci) of the bacteria, the formation of bacteriolysins with the possible setting free of endotoxins, possible vascular thrombosis and local abscess formation in vital organs. From this it will be seen, taking the death of the animal as the criterion, how difficult it is to say with such a complexity of factors, that the acquired immunity of an animal has any relation to one or all of the antibodies formed. In such acquired immunity against bacteria, not only the production of antibodies, but also phagocytosis, acquired resistance of vitally important cells such as those of the vital centres, the heart muscle &c. and accidental circumstances have to be considered in their due proportions, differing for each individual case. So that I take it that it is absolutely impossible from the essential nature of the circumstances, to attribute resistance to disease to/

to any one factor. This resistance to disease is due to a harmonious balancing of several factors which are at present quite beyond experimental control, and whose proportionate action in any given case consequently cannot be estimated. As a rider to this no prognostications should be made with regard to the ultimate termination of a bacterial infection from a consideration of one factor singly but only from a consideration of all the factors and their relation to one another.

SUMMARY AND CONCLUSIONS.

1. The method of investigating the seat of origin of antibodies, especially the haemolysins, bactericidal bodies and precipitins, by means of "Organ extracts" is valueless: and no reliance can be placed on results derived by such a method.

2. Normal rabbits, injected intravenously with ox blood corpuscles, show Immune body, in their blood, against ox blood corpuscles, for the first time, on or about the third day.

3. Normal rabbits, from which the spleen alone, the spleen and thyroid, the thyroid alone, or one kidney has been removed and into which, within a week or so after the operation, ox blood corpuscles have been injected intravenously, show, like the controls, Immune body, against ox blood corpuscles, for the first time on or about the third day.

4. The total leucocyte count of perfectly normal rabbits may vary in different individuals between/

All the rabbits referred are tame laboratory animals.

between roughly 4,000 and 15,000; in individual rabbits, in ordinary and exaggerated conditions of laboratory life, as regards feeding, time of day &c., the total leucocytes do not vary beyond 3,000 on either side of a mean per Cmm, that is beyond the limits to be allowed for experimental error.

5. The differential count, as regards Polymorphonuclears and lymphocytes, varies so much, under ordinary laboratory conditions, in the same rabbit, at different times, and in different rabbits, that conclusions should be drawn, with great caution, from any variation under experimental conditions.

6. The spleen was removed from a normal rabbit. Two days after its removal, it was injected intravenously with ox blood corpuscles. Immune body appeared in its blood in three days and yet there was no leucocytosis. To adopt the usual criterion this may be taken as evidence that a compensatory action on the part of the bone marrow and lymphatic apparatus has been excluded.

7/

7. Injections into normal rabbits, intravenously, intraperitoneally, and subcutaneously, of even large amounts of ox blood corpuscles do not produce a variation in the total leucocyte count beyond that in control animals.

8. Such injections, - and I speak here with the greatest caution - produce no effect on the differential count.

9. From the above results, I would conclude provisionally, that so far as such methods can settle such a question, there is great probability that neither, the spleen, nor the bone marrow, nor the lymphatic apparatus, nor the kidney, is the seat of formation in rabbits, of Immune body against ox blood corpuscles.

10. Normal rabbits, from whom se blood, a haemolysin, agglutinin and precipitin for ox blood has been carefully excluded, after being fed for some time on ox blood, show in their blood all of these active principles. This gives some support to the view, to be advanced in this paper, that the formation of antibodies is allied to the ordinary physiological processes of/

of assimilation: and as the liver is accredited by physiologists with a high rôle in such processes, to the liver being regarded provisionally as a possible seat of formation of Immune body,- for sensitised ox blood corpuscles.

11. The amount of complement in leech, citrate, fluoride, and oxalate plasmas of the rabbit is the same as in the corresponding leech, citrate, fluoride, and oxalate sera.

12. Normal aqueous humour of rabbits and the fluid from a tape worm cyst of the rabbit, do not contain complement for sensitised ox blood corpuscles. They are both highly specialised secretions.

13. The fluid that collects in a short time after puncture in the anterior chamber of the eye and Blister fluids do contain, such complement. They are both of the nature of transudates from the blood vessels.

14. As showing the highly specialised nature of the secretion in the anterior chamber of the eye, some rabbits, with a blood serum, of very high titre in Immune body against ox blood corpuscles, may have none of this Immune body in even/

even 0·5cc of their aqueous humour).

15. Normal rabbits may be bled to the extent of more than one third of their blood at one sitting, or of more than one half within 18 hours without showing any change in the complement content of their serum for sensitised ox blood corpuscles.

16. If a normal rabbit, whose blood has been ascertained to be free from Immune body against ox blood corpuscles, be injected intravenously with a large amount of ox blood corpuscles - 28cc equivalent to 56cc defibrinated blood - the ox blood corpuscles can be recognised in the rabbit's general circulation until about the third day. At that time there is a <u>critical</u> formation of Immune body, a <u>critical</u> disappearance of the ox blood corpuscles, and a <u>critical</u> haemoglobinuria. Contrary to what Sachs found, I did <u>not</u> observe any variation in the complement content for ox blood corpuscles in such an experiment.

17. The injection intravenously into normal rabbits of unsensitised ox blood corpuscles, even in/

in very large quantity, does not produce any diminution of complement. The injection intravenously into normal rabbits, of an amount of sensitised or saturated ox blood corpuscles calculated to be sufficient to use up all the complement in the rabbit's body produces no reduction of the complement, while the injection of <u>four times</u> the calculated amount of such corpuscles into a similar rabbit, produces a <u>scarcely perceptible</u> diminution of the complement. On the other hand, the injection intravenously into rabbits, which have been immunised against ox blood corpuscles, of a quantity of fresh, sensitised, or saturated corpuscles far from sufficient to use up all the calculated amount of complement in the animal's body, markedly reduces the complement content of the animal's blood. Such animals often die rapidly. The possible explanation of both Phenomena may be, that the sudden lysis sets free stromata, which absorb the complement and further block the capillaries of the lung and other organs.

18. If a normal rabbit be injected intraperitoneally with inactivated Immune serum against rabbit corpuscles, obtained by injecting a/

a guinea pig with rabbit corpuscles, this heated immune serum appears to be absorbed into the rabbit's blood, to find complement there free in the blood, to unite with it and to cause extracellular haemolysis of the rabbit's red blood corpuscles and intense haemoglobinuria.

19. There is great probability therefore, that complement exists, free in the circulating plasma of the blood, and that, further, the organ, producing complement, must be one of great metabolic activity and of exceptional powers of regeneration.

20. The extirpation of the spleen and of the thyroid in rabbits may be said to cause no variation in the complement content of rabbits' serum.

21. Taking such things in relation, with the positive findings of Nolf, Ehrlich &c., I submit that the liver ought to be considered as a possible seat of origin of complement.

22. When hen corpuscles are injected intravenously into rabbits, the corpuscles tend to accumulate and to be retained in the liver.

23/

23. Such corpuscles are phagocyted to a small degree by the liver cells and Kupfer cells. No evidence was observed for phagocytosis taking place in the spleen or any of the other organs.

24. The liver cells are capable of a wide variation in morphological appearance, possibly corresponding with a similar wide range of metabolic activity and functioning.

25. Taking into consideration the facts exclusive and presumptive accumulated above concerning the seat of origin of Immune body, the facts of the accumulation and retention of hen corpuscles in the liver and then phagocytosis there, and the fact of the great variation possible in the liver cell, I submit that the liver ought to be considered as a possible seat of origin of Immune body.

26. To generalise, I would submit the possibility of the formation of antibodies being, in nature, the hypertrophy of a normal physiological process - a process which physiologically deals with overflow from the intestinal tract of material/

material, toxic and non toxic, fluid and particulate, multiplying and non multiplying and which has escaped the process of intestinal digestion to which the bulk of absorbed material is subjected before absorption.

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BIBLIOGRAPHY.

1. ACHARD & BENSAUDE. Archiv. de Médécin, Exper. 8. 1896.
2. " " " " " " "
3. ARLOING. Semaine Medicale. 1898.
4. ASCOLI & RIVA. Münch. Medic. Woch. 1901.
5. ASCOLI. Deutsch. Med. Woch. 1902.
6. " Münch. Med. Woch. 1903.
7. BAIL. Cent. für Bakt. orig. Bd. 27 1900.
8. BASTIN. La Cellule, Tome 8. 1892.
9. BATELLI. C. R. Soc. Biol. 1904.
10. BAUMGARTEN. Cent. für Klin. Med. 1888 (Ref)
11. BELLEI. Münch. Med. Wochen. 1904.
12. VON BERGMANN & E. SAVINI. Zeitsch. für Exp. Path. und Therap. 4. 1907.
13. BIONDI. Vierteljahrt f. gerich. med-) 3 Folge icin und off. sanit.) Bd. 23.
14. BLUMREICH & JAKOBY. Zeitschrift für Hygiène. Bd. 29.
15. BORDET. Annal. de l'Inst. Past. Tome 9. 1895.
16. BRETON & PETIT. C. R. Soc. Biol. Tome 64. 1908.
17. " " " " " " " "
18. BRETON & MASSOL. " " " " " "
19. BREZINA. Wien. Klin. Wochen. 1905.
20. BRINKERHOFF & TYZZER. Jour. Med. Research. 1902.
21./

21. BROWNING & McKENZIE. Journ. Path. and Bact. Jan. 1909.

22. BUCHNER. Münch Med. Woch. 1894.

23. " " " " 1897.

24. BULLOCH. Central. für Bact. orig. Bd.29. 1901.

25. BUSSE. " " " " " 47. 1908.

26. CALMETTE & BRETON. C. R. Soc. Biol. 1908.

27. " " Semaine Medicale. Tome 22.

28. CANTACUZÈNE. Ann. Inst. Past. 1897.

29. " " " " 1902.

30. " " " " 1908.

31. " C. R. Soc. Biol. Tome 63. p.345. 1907.

32. " " " " " " p.393. "

33. " " " " " " p.429. "

34. CEAPARU. C. R. Soc. Biol. 1908.

35. CELLER & HAMBURGER. Wien. Klin. Wochen. 11. 1905.

36. COCA. Univ. Pennsyl. Med. Bull. Oct. Vol. 21. 1908.

37. COUSADI. Hofmeis Beit. z. Chem. Phys. 1901.

38. COURMONT. Semaine Medicale. 1897.

39. DAUBLER. Central. für Bakt. Bd. 25.

40. DELZENNE. Travaux de Phys. Univ. Montpelier. Ref. 1898.

41. DENYS & KAISIN. La Cellule Tome 9. 1893.

42. DEUTSCH. Ann. de l'Inst. Past. 1899.

43. DONATTI.& LANDSTEINER. Zeitschr. für Hyg. Bd. 43. 1903.

44./

44. DOUGLAS. Journal of Phys. Vol. 33. 1905-6.

45. DOYON & POLICARD. C.R.Soc. Biol. Tome 66. Feb.1909.

46. DOEMENY. Wien. Klin. Wochen. Nr. 40. 1902.

47. DREYER & WALKER. B. M. J. Jan.1909.

48. VON DUNGERN. Munch Med. Woch. 1899.

49. " " " " " 1900.

50. " " Die Anti. Körper. 1903.

51. " " " " " "

52. EASON. Jour. Path. and Bact. 1906.

53. EHRLICH. Deutsch Med. Woch. Nr. 32 & 44. 1891.

54. " Berlin Klin. Woch. 1900.

55. VAN EMDEN. Zeitschr. für Hyg. Bd. 30.

56. ERDELY. Zeitschr. für Phys. " 46 1905.

57. FALLOISE. Bull. de l'acad. Scien. Belg. 1903.

58. " " " " " 1905.

59. " " " " " 1905.

60. FALLOISE & DUBOIS. Archiv Intern. Ac. Phys. Tome 2. 1904.

61. FERNI. Zeitschr. fur Hys. Bd. 60. 1908.

62. FIGARI. Berlin Klin. Woch. Nr. 7. 1904.

63. FLEISCHMAN & MICHAELIS. Medic. Klinik. Nr. 1. 1906.

64. FLÜGGE Zeitschr. für Hygien. Bd. 4. 1888.

65. FODOR. Deutsch. Med. Wochen. 1886 & '87.

66. FODOR & RIGLER. Centr. für Bakt. Bd. 23

67. FORNARIO. C. R. Soc. Biol. 1908.

68. FRASER. B. M. J. 1895.

69./

69. FUTAKI & GRUBER. Munch Med. Wochen. Nr. 6. 1907.

70. GANGHOFNER. Munch Med. Woch. Nr.34. 1904.

71. GENGOU. Annal de l'Inst. Past. 1899.

72. " " " " 1901.

73. " " " " "

74. " " " " "

75. " " " " "

76. GOODALL, PATON & GULLAND. Jour. of Phys. Vol.30 1903
" 33 1905.

77. GRUBER & DURHAM. Wiener Med. Woch. 11 & 12. 1896.

78. GRUBER. Munch Med. Wochen. 46 - 49. 1901.

79. " " " " " "

80. " Wien. Klin. Woch. 1903.

81. HAHN. Archiv. für Hyg. Bd. 25. 1895.

82. HAMBURGER. Wien. Klin. Woch. 15. 1902.

83. HAVET. La Cellule. Tome 10.

84. " " " " "

85. HERMANN. Bull. acad. scien. Belg. Pax. 2. 1904.

86. HEWLETT. Archiv. für Expt. Path. und Pharm. Bd. 49. 1903.

87. HERRING & SIMPSON. Proc. Roy. Soc. Series B. Vol. 78. 1906.

88. HOFMEISTER. Archiv. für Expt. Path. u. Pharm. Bd.22. 1887.

89. HOKE. Central. für Bakt. Bd. 84. 1903.

90. HOLMES. Guy's Hosp. Reports. Vol. 59. 1905.

91./

91. HUNTER. Journal of Phys. Vol. 32 1905.

92. JAKUSCHAWITSCH. Zeit. für Hygiène. Bd.47. H.3.

93. JATTA. " " " " 33. 1900.

94. JOSEPH. Jour. Expt. Med. Vol. 11. 1909.

95. KLEIN. Wien. Klin. Woch. No. 52. 1901.

96. KORSHUN. Annal de l'Inst. Past. Tome 22. 1908.

97. KRAUS & LEVADITI. C.R. Acad. Science. 1904.

98. KRAUS & SCHIFFMAN. Wiener Klin. Wochen. Nr.40 1905.

99. " " Annal de l'Inst. Past. 1906.

100. LAMBOTTER. Central für Bakt. Bd. 34. 1903.

101. LAMBOTTER & STIENNON. Central für Bakt. orig. Bd. 40. H.2.

102. LANDSTEINER. Central für Bakt. orig. Bd. 25.

103. " Wiener Klin. Woch. No. 19. 1897.

104. LANDSTEINER & STANKOVITCH. Centr. für.Bakt. No. 42. 1906.

105. LASCHTSCHENKO. Archiv für Hyg. Bd. 37. 1900.

106. LEVADITI. Annal. Inst. Past. Tome 15. 1901.

107. " " " " " " "

108. " " " " " " "

109. " " " " " " 1902.

110. " " " " " " 1903-4.

111. " Lesort des Hematics nuclées &c. Bucharest Imprimerie de l'état. 1905.

112. " " " " " " "

113. " " " " " " "

114./

114. LISIN. Archiv Intern. de Pharm.& Therap. 1908.

115. LONDON. Archiv. des sciences Biol.(Russes) Ref. 1901.

116. " Archiv f. Biol. Wissenschaften. Ref. 1901.

117. LOWENSTEIN. Zeits. für Hyg. 1905.

118. LÖWITT. Zieglers Beit. Bd. 22. 1900.

119. LÖWITT & SCHWARZ. Zeitsch. f. Heilkunde. H.8. 1903.

120. LUBARSCH. Central für Bakt. 1889.

121. McGOWAN. Jour. Path. and Bact. Vol.12. 1908.

122. MAZZEI. Ann. Ig. Sper. 17 3. Ref.

123. " " " " " " "

124. METALNIKOFF. Central für Bakt. Bd. 29. 1901.

125. METCHNIKOFF. Annal Inst. Past. Tome 9. 1895.

126. " " " " " 9 - 10 1895-6.

127. " " " " " 13. 1899.

128. " Immunity in Inf. Dis. page 550 Eng. trans. 1905.

129. MICHAELIS & OPPENHEIMER. Archiv anat. und Phys. Phys. abt. Supp. 1902.

130. MIONI. C.R. Soc.Biol. Tome 55. 1903.

131. MORO. Munich Med. Wochen. Nr. 5. 1906.

132. MOXTER. Deutsch Med. Woch. 1899.

133. MUIR. Lancet. Vol. 2. 1903.

134. MUIR & BROWNING. Jour. Path. and Bact. 1909.

135. NAKANISTU. Munich Med. Wochen. 1899.

136. NEDDEN. Von Graefes Archiv. f. Ophth. Bd.65. 1907.

137./

137.	NEUFELD.	Arb. a. d. k. gesund.	Bd. 28.	1908.
138.	NISSEN.	Zeitschr. für Hyg.	Bd. 6.	1889.
139.	"	" " "	" "	"
140.	NOLF.	Bull. acad. Roy. Scienc.	Belg.	1908
141.	NUTTALL.	Jour. Trop. Med.	Vol. 4.	1901.
142.	PATON & GOODALL.	Journal of Phys.	Vol. 29.	1903.
143.	PETIT & MINET.	C. R. Soc. Biol.		1908.
144.	PETTERSON.	Archiv für Hyg.	Bd. 43	1902.
145.	"	" " "	" "	"
146.	"	Central für Bakt.	" 40	1906.
147.	PETRIE.	Jour. of Path. and Bact.	Vol. 9.	1903.
148.	PETRONE & PAGAUD.	La Presse Medicale.	Vol. 15.	1907.
149.	PFEIFFER.	Deutsche Med. Wochen.	7 & 8.	1896.
150.	"	Archiv f. Expt. Path. u. Pharm.	Bd. 50.	1904.
151.	"	Rapport XIII Congres. Hyg. Brux.		1903.
152.	PFEIFFER & MARX.	Zeitschr. für Hyg.	Bd. 27.	1898.
153.	PIRENNE.	Central für Bakt. orig.	Bd. 36.	1904.
154.	POHL.	Archiv f. Expt. Path. u. Pharm.	Bd. 25.	1889.
155.	PONFICK.	Virchow Archiv.	Vol. 48.	1869.
156.	RATH.	Central für Bakt.	Bd. 25.	
157.	REHNS.	C. R. Soc. Biol.		1901.
158.	REMY.	Annal Inst. Past.		1906.
159.	ROEMER.	Von Graefes Arch. f. Ophth.	Bd. 52.	1901.
160.	ROSENAU & ANDERSON.	Jour. Inf. Dis.	Vol. 5.	1908.

161./

161.	RUFFER & CRENDIROPOULO.	B. M. J.	Vol. 1.	1902.
162.	SACHS.	Archiv für Anat. u. Phys.		1903.
163.	SALIMBENI.	Annal Inst. Past.		1897.
164.	SAWTCHENKO.	" " "		"
165.	"	" " "		1902.
166.	SCHÄFER.	Proc. Roy. Soc. Edin.	Vol. 24.	1902.
167.	SCHATTENFROH.	Archiv für Hyg.	Bd. 35.	1899.
168.	SCHIBAYAMA.	Centr. für Bakt.	Bd. 30.	1901.
169.	SCHNEIDER. L.	Archiv für Hyg.	Bd. 28.	1897.
170.	" R.	" " "	" 64.	1908.
171.	" "	" " "	" "	"
172.	" "	München Med. Woch.		1908.
173.	SCHÜTZE & SCHELLER.	Zeitsch. f. Hygiène.	Bd. 36.	1901.
174.	SICK.	Deuts. Archiv für Klin. Med. Bd. 80. H. 3 & 4.		
175.	SIMNITZKY.	München Med. Wochen.		1903.
176.	SMITH. (HENDERSON)	Proc. Roy. Soc.	Series B. Vol. 79.	
177.	STEINBERG.	Wien. Klin. Woch.	Bd. 21.	1908.
178.	SWEET.	Cent. für Bakt.	" 33.	1903.
179.	SZEKELY & SZANA.	" " "	" 12.	1892.
180.	SZOKALSKY.	Medyeyna.	Reference.	1908.
181.	TARASSEVITCH.	Annal. Inst. Past.		1902.
182.	"	Dissertation, Odessa.	Reference	1902.
183.	TCHISTOVITCH.	Annal Inst. Past,	Tome 13.	1899.
184.	TCHITCHKINE.	" " "	" 18.	1904.
185.	/			

185.	TROMMSDORFF.	Archiv für Hygiène.	Bd. 40.	1901.
186.	"	Habiletationschrift München.	Ref.	1906.
187.	UHLENHUTH.	Deutsch. Med. Woch.	No. 46.	1900.
188.	WALKER.	Jour. of Hyg.	Vol. 3.	1903.
189.	WASSMÜTH.	Archiv für Hyg.		1907.
190.	WASSERMANN.	Zeitschr. für Hyg.	Bd. 37.	1901.
191.	"	Deutsch. Med. Woch.		1899.
192.	WASSERMANN & TAKAKI.	Berlin Klin. Wochen.	No. 10.	1898.
193.	WESSELY.	Deutsche Med. Wochen.	No.7 & 8.	1903.
194.	WEBER.	C. R. Soc. Biol.	Feb.	1909.
195.	WIDAL & SICARD.	Bull. acad. Med.	Sept.	1896.
196.	WILDE.	Zeitsch. für Hyg.	Bd. 37.	1901.
197.	WOLF.	Berlin Klin. Woch.	17 - 20	1903.

IN VIVO EXPERIMENTS WITH COMPLEMENT.

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IN VIVO EXPERIMENTS WITH COMPLEMENT.

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The following experiments were undertaken with the object of determining what change, if any, take place in the complement content of the serum of fresh and immunised rabbits (*v.* ox blood corpuscles) on the intravenous injection of unsensitised, sensitised,[2] and saturated ox blood corpuscles. Rabbits were bled from the ear vein to the amount of 15 c.c.; they were then injected with the ox blood corpuscles, and usually in one and a half hours, sometimes after an hour, blood was again obtained from them either from the ear vein or from the internal jugular. These samples were allowed to clot, and the serum of each was allowed to remain in contact with the clot for the same period of time, to have the conditions as nearly as possible comparable; and, as far as circumstances would allow, everything was done that was essential for a strict comparison of the sera before and after injection. What was aimed at in regard to the dosage of corpuscles was to give a dose sufficient to use up all the complement present in the animal's body; it was considered inexpedient to experimentally determine the amount of complement present in the animal to be used as a guide to the dose of corpuscles to be given, as this would have involved such an exhaustive bleeding of the animal, previous to the bleeding at the time of the experiment, as was bound to have an effect on the economy of the animal. It was thought that it would serve the purposes of the research if a dose of corpuscles, in round numbers judged to be about equal to that necessary to use up all the complement in the animal's body, was given. As the amount of complement varies in the serum of different rabbits, this dose was afterwards seen sometimes to be less than the actual amount ascertained by subsequent calculations, sometimes it was greater. In calculating after the experiment how much of the complement—supposing that the complement circulated free in the plasma—ought theoretically to have been used up in the experiment, the M.H.D. of

[1] The subject of a communication to the Pathological Society of Great Britain and Ireland, January 3–4, 1908. [Received for publication February 18, 1908.]

[2] Corpuscles can take up several multiples of the animal hæmolytic dose or sensitising dose; when corpuscles are treated with these multiples they are said to be saturated.

complement estimated in the serum of the animal "before injection" was used. The amount of blood in the animal's body was calculated from Douglas' (1905–6) figures, which give 4·85 per cent. of body weight of blood in the buck and 5·3 per cent. of body weight in the doe. Rabbit serum amounts to about 50 per cent. of the blood. This was determined by taking small tubes with 3 c.c. of blood and centrifugalising them with a hand centrifuge at 4000 revolutions per minute until the clot was deposited as far as it would go. From these data it could be estimated how much of the total complement of the animal's body—supposing that complement was a limited definite quantity circulating in the plasma—ought to have been used up by the dose of ox blood corpuscles injected.

EXPERIMENTS.

Series I.

To determine the change in complement content of the serum of a fresh rabbit after injection intravenously of *sensitised* ox blood corpuscles.

Experiment 1.

Rabbit, buck; weight, 2000 grms.; ox blood corpuscles were washed 6 times; and the M.H.D. of the heated immune body used to sensitise them was 0·012 c.c. of a 1 in 6 dilution for 1 c.c. 1 per cent. suspension. The rabbit was bled to the extent of 15 c.c., then immediately a mixture of 2 c.c. ox corpuscles + 2 c.c. 0·85 per cent. saline + 0·4 c.c. heated immune serum (sensitising dose calculated from the M.H.D. given above) was injected into the ear vein. This mixture had been kept at 37° C. for 1½ hour previous to injection. The animal showed no symptoms of distress after injection, and survived. One and a quarter hour after injection 15 c.c. of blood were again drawn from the ear vein. The sera "before" and "after" were allowed to separate, each remaining in contact with the clot for the same time. The sera were then decanted and the estimation of the complement undertaken. (To avoid repetition it may be said that the same routine was gone through in the other experiments.) The serum "after" injection was deeply hæmoglobin tinged.

Determination of Complement.

Complement was diluted 1 in 3 with saline (0·85 per cent.); heated immune serum was diluted 1 in 6 with saline 0·85 per cent.; M.H.D. for 1 c.c. 1 per cent. suspension was 0·012 c.c.; 1 per cent. suspension of ox blood corpuscles used.

Before Injection.

	1 per cent. Suspension O.B.C.		H.I.S.		Compt.		0·85 per cent. Saline.		
(1)	1 c.c.	+	0·012	+	0·6	+	0	=	++++[1]
(2)	1 „	+	0·012	+	0·55	+	0·05	=	++++
(3)	1 „	+	0·012	+	0·5	+	0·1	=	++++
(4)	1 „	+	0·012	+	0·45	+	0·15	=	++++
(5)	1 „	+	0·012	+	0·4	+	0·2	=	++
(6)	1 „	+	0·012	+	0·35	+	0·25	=	+
(7)	1 „	+	0·012	+	0	+	0·6	=	0

[1] ++++ = nearly complete hæmolysis.

After Injection.

	1 per cent. Suspension O.B.C.		H.I.S.		Compt.		0·85 per cent. Saline.		
(1)	1 c.c.	+	0·012	+	0·6	+	0	=	++++
(2)	1 „	+	0·012	+	0·55	+	0·05	=	++++
(3)	1 „	+	0·012	+	0·5	+	0·1	=	++++
(4)	1 „	+	0·012	+	0·45	+	0·15	=	++++
(5)	1 „	+	0·012	+	0·4	+	0·2	=	++
(6)	1 „	+	0·012	+	0·35	+	0·25	=	+
(7)	1 „	+	0·012	+	0	+	0·6	=	0

The mixtures were kept at 37° C. for 1½ hour.

In the serum obtained before (as well as after) injection the M.H.D. of complement could not be determined exactly—complete hæmolysis not having occurred in the above set of tubes, and there not being enough of serum left in either case to do a further set. But 0·6 c.c. of the 1 in 3 dilution of complement with 0·012 c.c. of the diluted heated immune serum produced almost complete hæmolysis in 1 c.c. of a 1 per cent. suspension of ox blood corpuscles, while 0·35 c.c. of the diluted complement produced a trace in a similar mixture. In the serum obtained after injection the series of tubes prepared in exactly the same way in every detail show exactly the same result. If we take 0·7 c.c. of the diluted complement as the M.H.D. for 1 c.c. 1 per cent. suspension of ox blood corpuscles, then the 2 c.c. of ox blood corpuscles injected ought to have used up 46 c.c. of complement; while, taking the blood volume as 4·85 per cent. of the body weight and the complement = 50 per cent. of the blood, the total complement in the rabbit must in reality have been about 48 c.c.

Experiment 2.

Rabbit, female; weight, 2220 grms.; ox blood corpuscles washed six times; M.H.D. of heated immune body to sensitise them was 0·01 c.c. of a 1 in 6 dilution for 2 c.c. of a 1 per cent. suspension of ox blood corpuscles. The rabbit was bled from the ear vein to the extent of 15 c.c.; and then immediately a mixture of 3 c.c. corpuscles + 0·24 c.c. heated immune serum + 2 c.c. saline, which had been kept at 37° C. for one and a half hour was injected into the ear vein. This animal again showed no symptoms of distress after injection, and one and a half hour after injection 15 c.c. blood were again drawn from the ear vein. The sera were allowed to separate as before, and the M.H.D. determined as before. The serum after injection was again darkly hæmoglobin tinged.

Determination of Complement.

Complement was undiluted; heated immune serum was diluted 1 in 6 with 0·85 per cent. saline. M.H.D. for 2 c.c. 1 per cent. suspension of ox blood corpuscles = 0·01 c.c.; 1 per cent. suspension of ox blood corpuscles was used.

BEFORE INJECTION.

	1 per cent. Suspension O.B.C.		H.I.S.		Compt.		0·85 per cent. Saline.		
(1)	2 c.c.	+	0·01	+	0·5	+	0	=	C.[1]
(2)	2 ,,	+	0·01	+	0·45	+	0·05	=	C.
(3)	2 ,,	+	0·01	+	0·4	+	0·1	=	C.
(4)	2 ,,	+	0·01	+	0·375	+	0·125	=	C.
(5)	2 ,,	+	0·01	+	0·35	+	0·15	=	C.
(6)	2 ,,	+	0·01	+	0·325	+	0·175	=	C.
(7)	2 ,,	+	0·01	+	0·3	+	0·2	=	C.
(8)	2 ,,	+	0·01	+	0·25	+	0·25	=	++++
(9)	2 ,,	+	0·01	+	0·2	+	0·3	=	++++
(10)	2 ,,	+	0·01	+	0	+	0·5	=	0

[1] C. = 1½ hour at 37° C.

AFTER INJECTION.

(1)	2 c.c.	+	0·01	+	0·5	+	0	=	C.
(2)	2 ,,	+	0·01	+	0·45	+	0·05	=	C.
(3)	2 ,,	+	0·01	+	0·4	+	0·1	=	C.
(4)	2 ,,	+	0·01	+	0·375	+	0·125	=	C.
(5)	2 ,,	+	0·01	+	0·35	+	0·15	=	C.
(6)	2 ,,	+	0·01	+	0·325	+	0·175	=	C.
(7)	2 ,,	+	0·01	+	0·3	+	0·2	=	C.
(8)	2 ,,	+	0·01	+	0·25	+	0·25	=	++++
(9)	2 ,,	+	0·01	+	0·2	+	0·3	=	++++
(10)	2 ,,	+	0·01	+	0	+	0·5	=	0

1½ hour at 37° C.

The M.H.D. of complement before injection for 2 c.c. 1 per cent. ox blood corpuscle suspension sensitised with 0·01 c.c. of a dilution of 1 in 6 heated immune serum is therefore 0·3 c.c., and the M.H.D. of complement after injection under the same circumstances is exactly the same; and the results of the two series of determinations is exactly the same in every detail. Here, if 0·3 c.c. complement is taken as the M.H.D. of complement for 2 c.c. of a 1 per cent. suspension of ox blood corpuscles, then the 3 c.c. ox blood corpuscles injection ought to have used up 45 c.c. complement, and, calculated as before, the animal being a doe, the total amount of complement present in its body amounted to 58 c.c.

SERIES II.

To determine the change in complement content of the serum of a fresh rabbit, after injection intravenously of ox blood corpuscles *sensitised to saturation*, the possibility of which was originally pointed out by Ehrlich and Morgenroth (1900). An example of the method of obtaining saturation of corpuscles may be here given.

The M.H.D. of heated immune serum for 2 c.c. 1 per cent. suspension of ox blood corpuscles is 0·05 c.c. of a 1 in 6 dilution when the mixture is fully complemented. One per cent. ox blood corpuscle suspension used. Complement added in excess undiluted.

	Ox Blood Corpuscle Suspension.		H.I.S.		Saline.		
(1)	2 c.c.	+	0·05 c.c.	(1 M.H.D.) +	0·25 c.c.		Incubated one and a half hour at 37° C.; then centrifugalised and supernatant fluid added to deposit from 2 c.c. 1 per cent. suspension of fresh ox blood corpuscle.
(2)	2 ,,	+	0·1 ,,	(2 ,,) +	0·20 ,,		
(3)	2 ,,	+	0·15 ,,	(3 ,,) +	0·15 ,,		
(4)	2 ,,	+	0·20 ,,	(4 ,,) +	0·1 ,,		
(5)	2 ,,	+	0·25 ,,	(5 ,,) +	0·05 ,,		
(6)	2 ,,	+	0·30 ,,	(6 ,,) +	0 ,,		

	Supernatant Fluid + Fresh Ox Blood Corpuscles.		Complement.		
(1)	2 c.c.	+	0·5 c.c.	=	0
(2)	2 ,,	+	0·5 ,,	=	0
(3)	2 ,,	+	0·5 ,,	=	+
(4)	2 ,,	+	0·5 ,,	=	+++
(5)	2 ,,	+	0·5 ,,	=	C.
(6)	2 ,,	+	0·5 ,,	=	C.

This mixture was kept in the incubator at 37° C. for one and a half hour. It will be seen that the corpuscles took up at least 2 M.H.D. of heated immune serum.

Experiment 1.

RABBIT, doe; weight, 1460 grms.; ox blood corpuscles washed six times. M.H.D. of the heated immune serum used was 0·05 of a 1 in 6 dilution for 2 c.c. 1 per cent. suspension of ox blood corpuscles. For saturation the corpuscles were given 3 M.H.D.

The rabbit was bled to the extent of 15 c.c. It was then injected intravenously with a mixture of 3·48 c.c. heated immune serum (= 3 M.H.D.) +2·8 c.c. corpuscles. The mixture had been kept in the incubator for one and a half hour and the whole mixture (heated immune serum and corpuscles) was injected. After injection the rabbit was quite lively, and showed no symptoms of distress. A sample of blood was removed again in one and a half hour. The sera were allowed to separate as before. The serum obtained after injection was deeply hæmoglobin tinted.

To determine the M.H.D. of complement. Complement was undiluted; heated immune serum was diluted 1 in 6; M.H.D. = 0·05 c.c., for 2 c.c. 1 per cent. suspension of ox blood corpuscles. One per cent. suspension of ox blood corpuscles used.

BEFORE INJECTION.

	1 per cent. Suspension O.B.C.		H.I.S.		Compt.		Saline.		
(1)	2 c.c.	+	0·05	+	0·5	+	0	=	+++
(2)	2 ,,	+	0·05	+	0·4	+	0·1	=	+++
(3)	2 ,,	+	0·05	+	0·35	+	0·15	=	++
(4)	2 ,,	+	0·05	+	0·3	+	0·2	=	++
(5)	2 ,,	+	0·05	+	0·25	+	0·25	=	+
(6)	2 ,,	+	0·05	+	0·2	+	0·3	=	0
(7)	2 ,,	+	0·05	+	0·15	+	0·35	=	0
(8)	2 ,,	+	0·05	+	0·1	+	0·4	=	0
(9)	2 ,,	+	0·05	+	0	+	0·5	=	0

1½ hour at 37° C.

After Injection.

	1 per cent. Suspension O.B.C.	H.I.S.	Compt.	Saline.	
(1)	2 c.c.	+ 0·05	+ 0·5	+ 0	= +++
(2)	2 „	+ 0·05	+ 0·4	+ 0·1	= +++
(3)	2 „	+ 0·05	+ 0·35	+ 0·15	= ++
(4)	2 „	+ 0·05	+ 0·3	+ 0·2	= ++
(5)	2 „	+ 0·05	+ 0·25	+ 0·25	= +
(6)	2 „	+ 0·05	+ 0·2	+ 0·3	= 0
(7)	2 „	+ 0·05	+ 0·15	+ 0·35	= 0
(8)	2 „	+ 0 05	+ 0·1	+ 0·4	= 0
(9)	2 „	+ 0·05	+ 0	+ 0·5	= 0

1½ hour at 37° C.

Thus in the serum obtained before injection the M.H.D. of complement is not arrived at in the extent of the experiment, but hæmolysis is seen to start with 0·25 c.c. complement. In the serum obtained after injection hæmolysis starts also with 0·25 c.c. complement, and the two series of results are exactly the same in every respect. Here, if 0·6 c.c. complement be taken as the M.H.D. of complement for 2 c.c. 1 per cent. ox blood corpuscle suspension, then the 2·8 c.c. of ox corpuscles injected ought to have used up 84 c.c. of the complement, and, calculated as before, the total amount of complement present in the animal's body only amounted to 38·6 c.c.

Experiment 2.

Rabbit, buck, weight, 2220 grms. Ox blood corpuscles were washed six times. M.H.D. of the heated immune serum was 0·01 c.c. of a 1 in 6 dilution for 1 c.c. of a 1 per cent. suspension. For saturation the corpuscles were given 6 M.H.D. of the heated immune serum.

The rabbit was bled to the extent of 15 c.c. It was then injected intravenously with a mixture of 4 c.c. heated immune serum (6 M.H.D.)+4 c.c. ox blood corpuscles which had been incubated for one and a half hour at 37° C. The rabbit showed great dyspnœa after injection, and was lying down in great distress. It recovered a little, and was living at the end of one and a half hour. At this time blood was obtained from the internal jugular. Post-mortem, there were a few hæmorrhages in the lung, and a few petechiæ subperitoneally. The urine contained abundant hæmoglobin. The corpuscular suspension used for injection contained minute flocculi visible to the naked eye, and this may explain the symptoms and post-mortem appearances. There was no air in the heart. The sera were allowed to separate as before. The serum after injection was deeply hæmoglobin tinted. The M.H.D. of the complement was then determined. In this determination complement was used undiluted; heated immune serum was diluted 1 in 6, of which M.H.D. = 0·01 c.c. for 1 c.c. 1 per cent. suspension of ox blood corpuscles; 1 per cent. suspension of ox blood corpuscles used.

Complement before Injection.

	1 per cent. Suspension O.B.C.	H.I.S.	Compt.	Saline.	
(1)	1 c.c.	+ 0·01	+ 0·4	+ 0	= C.
(2)	1 „	+ 0·01	+ 0·35	+ 0·05	= C.
(3)	1 „	+ 0·01	+ 0·03	+ 0·01	= C.
(4)	1 „	+ 0·01	+ 0·25	+ 0·15	= C.
(5)	1 „	+ 0·01	+ 0·02	+ 0·02	= C.
(6)	1 „	+ 0·01	+ 0·15	+ 0·25	= C.
(7)	1 „	+ 0·01	+ 0·1	+ 0·3	= C.
(8)	1 „	+ 0·01	+ 0·05	+ 0·35	= ++++
(9)	1 „	+ 0·01	+ 0·025	+ 0·375	= +
(10)	1 „	+ 0·01	+ 0	+ 0·4	= 0

1½ hour at 37° C.

After Injection.

(1)	1 „	+ 0·01	+ 0·4	+ 0	= C.
(2)	1 „	+ 0·01	+ 0·35	+ 0·05	= C.
(3)	1 „	+ 0·01	+ 0·3	+ 0·1	= C.
(4)	1 „	+ 0·01	+ 0·25	+ 0·15	= C.
(5)	1 „	+ 0·01	+ 0·2	+ 0·2	= C.
(6)	1 „	+ 0·01	+ 0·15	+ 0·25	= C.
(7)	1 „	+ 0·01	+ 0·1	+ 0·3	= C.
(8)	1 „	+ 0·01	+ 0·05	+ 0·35	= ++++
(9)	1 „	+ 0·01	+ 0·025	+ 0·375	= +
(10)	1 „	+ 0·01	+ 0	+ 0·4	= 0

1½ hour at 37° C.

Thus in the serum obtained before injection the M.H.D. of complement for 1 c.c. 1 per cent. suspension of ox blood corpuscles sensitised with 0·01 c.c. of the diluted treated immune serum is 0·1 c.c. In the serum obtained after injection the M.H.D. of the complement is the same. Here, if we take 0·1 c.c. complement as the M.H.D. of complement for 1 c.c. of 1 per cent. suspension of ox blood corpuscles, then the 4 c.c. corpuscles injected ought to have used up 40 c.c. complement, and the amount of complement present in the blood of the rabbit calculated as before is 53·8 c.c.

Series III.

To determine the change, if any, in complement content of the serum of a fresh rabbit after injection intravenously of *unsensitised* ox blood corpuscles.

Experiment 1.

Rabbit, buck; weight, 2320. Its serum contained no lysin for ox blood corpuscles. The rabbit was bled to the extent of 15 c.c. It was then injected intravenously with 4 c.c. ox blood corpuscles + 2 c.c. saline. It showed no symptoms of distress, and one and a half hour after injection it was bled again to the extent of 15 c.c. The sera were separated as before. The serum obtained after injection had only the slightest hæmoglobin tint.

Determination of the M.H.D. of Complement.

Complement was used undiluted; heated immune serum was diluted 1 in 6 M.H.D. for 2 c.c. One per cent. suspension of ox blood corpuscles = 0·06 of a suspension 1 in 6 dilution. One per cent. suspension of ox blood corpuscles used.

BEFORE INJECTION.

	1 per cent. Suspensions O.B.C.	H.I.S.	Compt.	Saline.	
(1)	2 c.c.	+ 0·06	+ 0·5	+ 0	= C.
(2)	2 ,,	+ 0·06	+ 0·45	+ 0·05	= C.
(3)	2 ,,	+ 0·06	+ 0·4	+ 0·1	= C.
(4)	2 ,,	+ 0·06	+ 0·35	+ 0·15	= C.
(5)	2 ,,	+ 0·06	+ 0·3	+ 0·2	= C.
(6)	2 ,,	+ 0·06	+ 0·25	+ 0·25	= C.
(7)	2 ,,	+ 0·06	+ 0·2	+ 0·3	= C.
(8)	2 ,,	+ 0·06	+ 0·15	+ 0·35	= ++++
(9)	2 ,,	+ 0·06	+ 0·1	+ 0·4	= +++
(10)	2 ,,	+ 0·06	+ 0	+ 0·5	= 0

1½ hour at 37° C.

AFTER INJECTION.

(1)	2 c.c.	+ 0·06	+ 0·5	+ 0	= C.
(2)	2 ,,	+ 0·06	+ 0·45	+ 0·05	= C.
(3)	2 ,,	+ 0·06	+ 0·4	+ 0·1	= C.
(4)	2 ,,	+ 0·06	+ 0·35	+ 0·15	= C.
(5)	2 ,,	+ 0·06	+ 0·3	+ 0·2	= C.
(6)	2 ,,	+ 0·06	+ 0·25	+ 0·25	= C.
(7)	2 ,,	+ 0·06	+ 0·2	+ 0·3	= C.
(8)	2 ,,	+ 0·06	+ 0·15	+ 0·35	= ++++
(9)	2 ,,	+ 0·06	+ 0·1	+ 0·4	= +++
(10)	2 ,,	+ 0·06	+ 0	+ 0·5	= 0

1½ hour at 37° C.

Thus in the serum obtained before injection the M.H.D. of complement for 2 c.c. 1 per cent. suspension of ox blood corpuscles is 0·2 c.c., and in the serum obtained after injection the M.H.D. is the same. Here, if we take 0·2 c.c. complement as the M.H.D. of complement for 2 c.c. 1 per cent. suspension of ox blood corpuscles, then the 4 c.c. ox blood corpuscles injected might have used up 40 c.c. complement, and the amount of complement present in the blood of the rabbit calculated as before amounts to 56 c.c.

Experiment 2.

RABBIT, buck; weight, 1900 grms. Serum contains normally no lysin for ox blood corpuscles. The rabbit was bled to the extent of 15 c.c. It was then injected intravenously with 3 c.c. ox blood corpuscles + 2 c.c. saline. It showed no signs of distress. One and a half hour after injection it was bled again to the extent of 15 c.c. The sera were then separated as before. The serum obtained after injection had only the slightest hæmogoblin tint.

Determination of the M.H.D. of Complement.

Complement undiluted. Heated immune serum was diluted 1 in 6; of which M.H.D. for 2 c.c. 1 per cent. suspension of ox blood corpuscles = 0·06 c.c. One per cent. suspension of ox blood corpuscles used.

BEFORE INJECTION.

	1 per cent. Suspension O.B.C.	H.I.S.	Compt.	Saline.	
(1)	2 c.c.	+ 0·06	+ 0·5	+ 0	= C.
(2)	2 ,,	+ 0·06	+ 0·45	+ 0·05	= C.
(3)	2 ,,	+ 0·06	+ 0·4	+ 0·1	= C.
(4)	2 ,,	+ 0·06	+ 0·35	+ 0·15	= C.
(5)	2 ,,	+ 0·06	+ 0·3	+ 0·2	= C.
(6)	2 ,,	+ 0·06	+ 0·25	+ 0·25	= C.
(7)	2 ,,	+ 0·06	+ 0·2	+ 0·3	= ++
(8)	2 ,,	+ 0·06	+ 0·15	+ 0·35	= +?[1]
(9)	2 ,,	+ 0·06	+ 0·1	+ 0·4	= +?
(10)	2 ,,	+ 0·06	+ 0	+ 0·5	= 0

1½ hour at 37° C.

AFTER INJECTION.

(1)	2 c.c.	+ 0·06	+ 0·5	+ 0	= C.
(2)	2 ,,	+ 0·06	+ 0·45	+ 0·05	= C.
(3)	2 ,,	+ 0·06	+ 0·4	+ 0·1	= C.
(4)	2 ,,	+ 0·06	+ 0·35	+ 0·15	= C.
(5)	2 ,,	+ 0·06	+ 0·3	+ 0·2	= C.
(6)	2 ,,	+ 0·06	+ 0·25	+ 0·25	= C.
(7)	2 ,,	+ 0·06	+ 0·2	+ 0·3	= ++
(8)	2 ,,	+ 0·06	+ 0·15	+ 0·35	= +?[1]
(9)	2 ,,	+ 0·06	+ 0·1	+ 0·4	= +?
(10)	2 ,,	+ 0·06	+ 0	+ 0·5	= 0

1½ hour at 37° C.

Thus in the serum obtained before injection the M.H.D. of complement for 2 c.c. 1 per cent. suspension of ox blood corpuscles is 0·25 c.c. In the serum obtained after injection the M.H.D. is the same. Here, if we take 0·25 c.c. as the M.H.D. of complement for 2 c.c. 1 per cent. suspension of ox blood corpuscles, then the 3 c.c. corpuscles injected might have used up 37·5 c.c. complement; and the amount of complement present in the blood of the rabbit calculated as before amounts to 46 c.c.

SERIES IV.

To determine the change in complement content of the serum of an *immune* rabbit after injection intravenously of *completely saturated* ox blood corpuscles

In determining the M.H.D. of the complement in the immune rabbit, after the injection of corpuscles the ox blood corpuscles

[1] Trace of hæmolysis doubtful.

were saturated with heated immune body (as compared with their being merely sensitised, as was done in the fresh rabbits). This was in order to make them independent of the immune body in the serum whose complement was to be determined. The other steps were the same as for the determination of M.H.D. of complement in fresh rabbits.

Experiment 1.

RABBIT, buck; weight, 2150 grms. This rabbit had been immunised for several weeks, and its serum contained immune body to such an amount that 0·005 c.c. when fully complemented hæmolysed completely 2 c.c. of a 1 per cent. suspension of ox blood corpuscles. The heated immune serum used for saturating the corpuscles had a M.H.D. for 2 c.c. 1 per cent. suspension of ox blood corpuscles of 0·01 c.c.; for saturation the corpuscles took up 3 M.H.D. The rabbit was bled to the extent of 15 c.c. It was then injected intravenously with a mixture of 4·5 c.c. heated immune serum (= 3 M.H.D.) + 3 c.c. ox blood corpuscles. The mixture had been kept in the incubator for one and a half hour at 37° C. The rabbit was very ill after injection and was dying when, after one and a half hour, blood was obtained from its jugular. The serum was allowed to separate as before. The serum after injection was darkly hæmoglobin stained.

Determination of the M.H.D. of Complement.

Complement was undiluted. Heated immune serum was diluted 1 in 6. M.H.D. = 0·06 for 2 c.c. 1 per cent. suspension of ox blood corpuscles. Three M.H.D. used to each tube to saturate the corpuscles and make them independent of the immune body in the serum whose complement was to be determined.

BEFORE INJECTION.

	1 per cent. Suspension O.B.C.		H.I.S.		Compt.		Saline.		
(1)	2 c.c.	+	0·18	+	0·5	+	0	=	C.
(2)	2 ,,	+	0·18	+	0·45	+	0·05	=	C.
(3)	2 ,,	+	0·18	+	0·4	+	0·1	=	C.
(4)	2 ,,	+	0·18	+	0·375	+	0·125	=	C.
(5)	2 ,,	+	0·18	+	0·35	+	0·15	=	C.
(6)	2 ,,	+	0·18	+	0·32	+	0·175	=	C.
(7)	2 ,,	+	0·18	+	0·3	+	0·2	=	C.
(8)	2 ,,	+	0·18	+	0·25	+	0·25	=	C.
(9)	2 ,,	+	0·18	+	0·2	+	0·3	=	C.
(10)	2 ,,	+	0·18	+	0	+	0·5	=	0
(11)	2 ,,	+	0·18	+	0·15	+	0	=	C.
(12)	2 ,,	+	0·18	+	0·1	+	0·05	=	+ + + +
(13)	2 ,,	+	0·18	+	0·09	+	0·06	=	+ + + +
(14)	2 ,,	+	0·18	+	0·08	+	0·07	=	+ + + +
(15)	2 ,,	+	0·18	+	0·07	+	0·08	=	+ + + +
(16)	2 ,,	+	0·18	+	0·06	+	0·09	=	+ + + +
(17)	2 ,,	+	0·18	+	0·05	+	0·1	=	+ + +
(18)	2 ,,	+	0·18	+	0·04	+	0·11	=	+ + +
(19)	2 ,,	+	0·18	+	0·03	+	0·12	=	+ ?
(20)	2 ,,	+	0·18	+	0	+	0·15	=	0

1½ hour at 37° C.

AFTER INJECTION.

	1 per cent. Suspension O.B.C.		H.I.S.		Compt.		Saline.		
(1)	2 c.c.	+	0·18	+	0·6	+	0	=	+ + +
(2)	2 ,,	+	0·18	+	0·5	+	0·1	=	+ + +
(3)	2 ,,	+	0·18	+	0·4	+	0·2	=	+ +
(4)	2 ,,	+	0·18	+	0·3	+	0·3	=	+ +
(5)	2 ,,	+	0·18	+	0·2	+	0·4	=	+
(6)	2 ,,	+	0·18	+	0·15	+	0	=	+
(7)	2 ,,	+	0·18	+	0·1	+	0·05	=	?
(8)	2 ,,	+	0·18	+	0·09	+	0·06	=	?
(9)	2 ,,	+	0·18	+	0·08	+	0·07	=	?
(10)	2 ,,	+	0·18	+	0·07	+	0·08	=	0
(11)	2 ,,	+	0·18	+	0·06	+	0·09	=	0
(12)	2 ,,	+	0·18	+	0·05	+	0·01	=	0
(13)	2 ,,	+	0·18	+	0·04	+	0·11	=	0
(14)	2 ,,	+	0·18	+	0·03	+	0·12	=	0
(15)	2 ,,	+	0·18	+	0	+	0·15	=	0

1½ hour at 37° C.

Thus in the serum obtained before injection the M.H.D. of complement for 2 c.c. 1 per cent. suspension of ox blood corpuscles is 0·15 c.c.; while in the serum after injection 0·6 c.c. of complement does not produce nearly complete hæmolysis in a like quantity of the suspension. Here, if we take 0·15 c.c. as the M.H.D. of complement for 2 c.c. 1 per cent. suspension of ox blood corpuscles, then the 3 c.c. corpuscles ought to have used up 22·5 c.c. of the complement; and the amount of complement present in the blood of the rabbit calculated as before amounts to 52 c.c.

Experiment 2.

RABBIT, buck; weight, 2250 grms. This rabbit's serum contained immune body to such an extent that 0·01 c.c. serum when fully complemented completely hæmolysed 2 c.c. 1 per cent. suspension of ox blood corpuscles. For saturation the corpuscles took up 3 M.H.D. of heated immune serum. The rabbit was bled to the extent of 15 c.c. Then a mixture of 4 c.c. ox corpuscles + 6 c.c. heated immune serum (3 M.H.D.) was injected intravenously. The mixture had been kept one and a half hour in the incubator. The rabbit had great dyspnœa and distress, and died a few minutes before the expiration of the one and a half hour it was to be allowed to live. Blood was obtained from the heart. Post-mortem it showed a large infarction of the small intestine. The serum was allowed to separate as before. The serum obtained after injection was darkly hæmoglobin tinted.

Determination of the M.H.D. of Complement.

Complement was undiluted. Heated immune serum was diluted 1 in 6; 3 M.H.D. were used to saturate the corpuscles to make them independent of the immune body in the serum whose complement was to be determined. M.H.D. of immune body for 2 c.c. 1 per cent. suspension of ox blood corpuscles = 0·06 of a 1 in 6 dilution.

COMPLEMENT BEFORE INJECTION.

	1 per cent. Suspension O.B.C.	H.I.S.	Compt.	Saline.	
(1)	2 c.c.	+ 0·18	+ 0·5	+ 0	= C.
(2)	2 ,,	+ 0·18	+ 0·4	+ 0·1	= C.
(3)	2 ,,	+ 0·18	+ 0·3	+ 0·2	= C.
(4)	2 ,,	+ 0·18	+ 0·2	+ 0·3	= C.
(5)	2 ,,	+ 0·18	+ 0·1	+ 0·4	= C.
(6)	2 ,,	+ 0·18	+ 0·08	+ 0·42	= ++++
(7)	2 ,,	+ 0·18	+ 0·06	+ 0·44	= ++
(8)	2 ,,	+ 0·18	+ 0·04	+ 0·46	= +
(9)	2 ,,	+ 0·18	+ 0·01	+ 0·49	= 0
(10)	2 ,,	+ 0·18	+ 0	+ 0·5	= 0

1½ hour at 37° C.

COMPLEMENT AFTER INJECTION.

(1)	2 c.c.	+ 0·18	+ 0·5	+ 0	= +++
(2)	2 ,,	+ 0·18	+ 0·4	+ 0·1	= ++
(3)	2 ,,	+ 0·18	+ 0·3	+ 0·2	= ++
(4)	2 ,,	+ 0·18	+ 0·2	+ 0·3	= +
(5)	2 ,,	+ 0·18	+ 0·1	+ 0·4	= 0
(6)	2 ,,	+ 0·18	+ 0·08	+ 0·42	= 0
(7)	2 ,,	+ 0·18	+ 0·06	+ 0·44	= 0
(8)	2 ,,	+ 0·18	+ 0·04	+ 0·46	= 0
(9)	2 ,,	+ 0·18	+ 0·01	+ 0·49	= 0
(10)	2 ,,	+ 0·18	+ 0	+ 0·5	= 0

1½ hour at 37° C.

Thus in the serum obtained before injection the M.H.D. of complement for 2 c.c. 1 per cent. suspension of ox blood corpuscles is 0·1 c.c. while in the serum obtained after injection 0·5 c.c. complement produced practically only about half hæmolysis. Here, if we take 0·1 c.c. as the M.H.D. of complement for 2 c.c. 1 per cent. suspension of ox blood corpuscles, then the 4 c.c. ox corpuscles injected ought to have used up 20 c.c. of complement; and the amount of complement present in the blood of the rabbit, calculated as before, amounts to 54 c.c.

SERIES V.

To determine the change in complement content of an *immune* rabbit after intravenous injection of fresh unsensitised ox blood corpuscles.

Experiment 1.

RABBIT, buck; weight, 2130 grms. This rabbit had been immunised for about six months with ox blood corpuscles. The M.H.D. of its immune body for 2 c.c. 1 per cent. suspension of ox blood corpuscles fully complemented was 0·0025 c.c.

The rabbit was bled to the extent of 15 c.c. Then 4·5 c.c. ox blood corpuscles + 2 c.c. saline, warmed in the incubator, were injected into the ear vein. The rabbit died in five minutes. Post-mortem there was no air found in the heart and no lesion was seen in any of the organs. The liver, lung, spleen, and kidney were examined microscopically and no signs of infarction seen. Blood was obtained from the heart. The sera were separated as before. The serum after injection was deeply hæmoglobin tinted.

Determination of the M.H.D. of Complement.

Complement was undiluted; heated immune serum was diluted 1 in 10, and 3 M.H.D. were used to saturate corpuscles to make them independent of the immune body introduced with the complement. M.H.D. of immune body = 0·025 c.c. of 1 in 10 dilution for 1 c.c. 1 per cent. supension. One per cent. suspension of ox blood corpuscles used.

COMPLEMENT BEFORE INJECTION.

	1 per cent. Suspension O.B.C.	H.I.S.	Compt.	Saline.	
(1)	1 c.c.	+ 0·075	+ 0·5	+ 0	= C.
(2)	1 ,,	+ 0·075	+ 0·4	+ 0·1	= C.
(3)	1 ,,	+ 0·075	+ 0·3	+ 0·2	= C.
(4)	1 ,,	+ 0·075	+ 0·2	+ 0·3	= C.
(5)	1 ,,	+ 0·075	+ 0·1	+ 0·4	= C.
(6)	1 ,,	+ 0·075	+ 0·08	+ 0·42	= C.
(7)	1 ,,	+ 0·075	+ 0·06	+ 0·44	= C.
(8)	1 ,,	+ 0·075	+ 0·04	+ 0·46	= C.
(9)	1 ,,	+ 0·075	+ 0·01	+ 0·49	= +
(10)	1 ,,	+ 0·075	+ 0	+ 0·5	= 0

1½ hour at 37° C.

COMPLEMENT AFTER INJECTION.

(1)	1 c.c.	+ 0·075	+ 0·5	+ 0	= +
(2)	1 ,,	+ 0·075	+ 0·4	+ 0·1	= +
(3)	1 ,,	+ 0·075	+ 0·3	+ 0·2	= +
(4)	1 ,,	+ 0·075	+ 0·2	+ 0·3	= +
(5)	1 ,,	+ 0·075	+ 0·1	+ 0·4	= +
(6)	1 ,,	+ 0·075	+ 0·08	+ 0·42	= +
(7)	1 ,,	+ 0·075	+ 0·06	+ 0·44	= +
(8)	1 ,,	+ 0·075	+ 0·04	+ 0·46	= 0
(9)	1 ,,	+ 0·075	+ 0·01	+ 0·49	= 0
(10)	1 ,,	+ 0·075	+ 0	+ 0·5	= 0

1½ hour at 37° C.

Thus in the serum obtained before injection the M.H.D. of complement was 0·04 c.c. for 1 c.c. 1 per cent. suspension, while in the serum after injection 0·5 c.c. complement produced only a trace of hæmolysis. Here, if we take 0·04 c.c. as the M.H.D. of complement for 1 c.c. 1 per cent. suspension of ox blood corpuscles, then the 4·5 c.c. corpuscles injected ought to have used up 18 c.c. complement; and the amount of the complement in the blood of the rabbit, calculated as before, amounts to 51 c.c.

Experiment 2.

RABBIT, buck; weight, 2370 grms. This rabbit's serum contains immune body to such an extent that 0·07 of a 1 in 10 dilution when fully complemented hæmolysed 1 c.c. of a 1 per cent. suspension of ox blood corpuscles.

The rabbit was bled to the extent of 15 c.c., it was then injected with 4·5 c.c. ox blood corpuscles + 2 c.c. saline which had been warmed in the incubator. It became very ill two minutes after injection with great dyspnœa, got gradually better, and when killed was almost recovered. Post-mortem, no hæmorrhages were seen anywhere. Hæmoglobin was present in the urine. The sera were allowed to separate as before. The serum obtained after injection was darkly hæmoglobin tinted.

Determination of the M.H.D. of Complement.

Complement was undiluted. Immune body undiluted, and about four times M.H.D. added to corpuscles to saturate them; *i.e.* 0·03 c.c. of the undiluted serum added to 1 c.c. 1 per cent. suspension of ox blood corpuscles. One per cent. suspension of ox blood corpuscles used.

COMPLEMENT BEFORE INJECTION.

	1 per cent. Suspension O.B.C.		H.I.S.		Compt.		Saline.		
(1)	1 c.c.	+	0·03	+	0·5	+	0	=	C.
(2)	1 „	+	0·03	+	0·4	+	0·1	=	C.
(3)	1 „	+	0·03	+	0·3	+	0·2	=	C.
(4)	1 „	+	0·03	+	0·2	+	0·3	=	C.
(5)	1 „	+	0·03	+	0·1	+	0·4	=	C.
(6)	1 „	+	0·03	+	0·08	+	0·42	=	++++
(7)	1 „	+	0·03	+	0·06	+	0·44	=	+++
(8)	1 „	+	0·03	+	0·04	+	0·46	=	+
(9)	1 „	+	0·03	+	0·01	+	0·49	=	0
(10)	1 „	+	0·03	+	0	+	0·5	=	0

1½ hour at 37° C.

COMPLEMENT AFTER INJECTION.

(1)	1 c.c.	+	0·03	+	0·5	+	0·0	=	C
(2)	1 „	+	0·03	+	0·4	+	0·1	=	C.
(3)	1 „	+	0·03	+	0·3	+	0·2	=	C.
(4)	1 „	+	0·03	+	0·2	+	0·3	=	C.
(5)	1 „	+	0·03	+	0·1	+	0·4	=	+
(6)	1 „	+	0·03	+	0·08	+	0·42	=	0
(7)	1 „	+	0·03	+	0·06	+	0·44	=	0
(8)	1 „	+	0·03	+	0·04	+	0·46	=	0
(9)	1 „	+	0·03	+	0·01	+	0·49	=	0
(10)	1 „	+	0·03	+	0	+	0·5	=	0

1½ hour at 37° C.

Thus in the serum obtained before injection the M.H.D. of complement was 0·1 c.c. for 1 c.c. 1 per cent. suspension of ox blood corpuscles, and there was a trace of hæmolysis with 0·04 c.c.; while in the serum after injection the hæmolysis was nearly complete with 0·2 c.c. complement for a like quantity of ox blood corpuscles, while with 0·1 c.c. there was only a trace, and with 0·08, 0·06, and 0·04 there was no hæmolysis. Here, if we take 0·1 c.c. as the M.H.D. for 1 c.c. 1 per cent. suspension of ox blood corpuscles, then the 4 c.c. ox blood corpuscles injected ought to have used up 45 c.c. complement,

and the amount of complement present in the blood of the rabbit calculated as before amounts to 57 c.c.

It will be well here (1) to bring forward evidence that the change in the complementing action of the serum in Series IV. and V. is really due to a diminution in complement, and not due to some action produced *in vivo* by the large excess of immune body in the complementing serum; (2) to show by an experiment how the minimal hæmolytic dose of complement is much lowered by adding *in vitro* an excess of immune body.

As regards (1)—that the change in the complementing power of the serum is due to a diminution in complement—this cannot be proved directly, but indirect proof can be got by determining the complement content of the blood of a fresh rabbit before and after injection of saturated corpuscles, and by using in this determination a large excess of heated immune body. It will be seen that the complement content of the rabbit's blood is exactly the same before and after injection—a condition which was also found in Series II., where a fresh rabbit was similarly injected, and where only one M.H.D. of heated immune body was used in the determination of the complement before and after injection.

Experiment.

RABBIT, buck; weight, 1850 grms. Bled 10 c.c. at 10.30; then injected at 10.40 intravenously with 3 c.c. ox blood corpuscles + 1·35 c.c. heated immune serum, the mixture having been in incubator at 37° C. for one and a half hour. (M.H.D. of heated immune serum for 1 c.c. 1 per cent. suspension of ox blood corpuscles = 0·0015 heated immune serum; here the corpuscles were saturated with 3 M.H.D.) Blood obtained after injection at 12.10; serum separated as before; serum after injection deeply hæmoglobin tinted.

Determination of the Complement.

Complement was undiluted. Heated immune serum (whose M.H.D. for 1 c.c. 1 per cent. suspension of ox blood corpuscles = 0·0015 c.c.) used in a dilution of 1 in 20 and in dose of 3 M.H.D. for sensitising to saturation the corpuscles, and used full strength in doses of 0·3 c.c. for the purpose of adding great excess of immune body to the various test mixtures. One per cent. ox blood corpuscles used.

BEFORE INJECTION.

	1 per cent. O.B.C.		H.I.S. 1 in 20.		Compt.		H.I.S. undil.		Saline.		
(1)	1 c.c.	+	0·09 c.c.	+	0·5 c.c.	+	0·3 c.c.	+	0	=	C.
(2)	1 „	+	0·09 „	+	0·4 „	+	0·3 „	+	0·1	=	C.
(3)	1 „	+	0·09 „	+	0 3 „	+	0·3 „	+	0·2	=	C.
(4)	1 „	+	0·09 „	+	0·2 „	+	0·3 „	+	0·3	=	C.
(5)	1 „	+	0·09 „	+	0·1 „	+	0·3 „	+	0·4	=	C.
(6)	1 „	+	0·09 „	+	0·08 „	+	0·3 „	+	0·42	=	C.
(7)	1 „	+	0·09 „	+	0·06 „	+	0·3 „	+	0·44	=	C.
(8)	1 „	+	0·09 „	+	0·04 „	+	0·3 „	+	0·46	=	C.
(9)	1 „	+	0·09 „	+	0·02 „	+	0·3 „	+	0·48	=	+++
(10)	1 „	+	0·09 „	+	0 „	+	0·3 „	+	0·5	=	0

1½ hour at 37° C.

After Injection.

	1 per cent. O.B.C.		H.I.S. 1 in 20.		Compt.		H.I.S.		Saline.	
(1)	1 c.c.	+	0·09 c.c.	+	0·5 c.c.	+	0·3 c.c.	+	0	= C.
(2)	1 „	+	0·09 „	+	0·4 „	+	0·3 „	+	0·1	= C.
(3)	1 „	+	0·09 „	+	0·3 „	+	0·3 „	+	0·2	= C.
(4)	1 „	+	0·09 „	+	0·2 „	+	0·3 „	+	0·3	= C.
(5)	1 „	+	0·09 „	+	0·1 „	+	0·3 „	+	0·4	= C.
(6)	1 „	+	0·09 „	+	0·08 „	+	0·3 „	+	0·42	= C.
(7)	1 „	+	0·09 „	+	0·06 „	+	0·3 „	+	0·44	= C.
(8)	1 „	+	0·09 „	+	0·04 „	+	0·3 „	+	0·46	= C.
(9)	1 „	+	0·09 „	+	0·02 „	+	0·3 „	+	0·48	= +++
(10)	1 „	+	0·09 „	+	0 „	+	0·3 „	+	0·5	= 0

1½ hour at 37° C.

Here it will be seen that, in spite of the great excess of heated immune serum (of which 2 c.c.+2 c.c. 1 per cent. ox blood corpuscles mixed and incubated gave no hæmolysis in one and a half hour), there is no change in the complement content after injection, thus showing that the large excess of immune body does not affect an accurate comparative estimation of the amount of complement.

(2) The following experiment shows the great lowering of the M.H.D. of complement when excess of heated immune serum is added:—

Complement Undiluted.

(1) *Without Excess of Heated Immune Serum.*

	1 per cent. O.B.C.		H.I.S. 1 in 10.		Saline.		Compt.		Saline.	
(1)	1 c.c.	+	0·08 c.c.	+	0·3 c.c.	+	0·5	+	0	= C.
(2)	1 „	+	0·08 „	+	0·3 „	+	0·4	+	0·1	= C.
(3)	1 „	+	0·08 „	+	0·3 „	+	0·3	+	0·2	= C.
(4)	1 „	+	0·08 „	+	0·3 „	+	0·2	+	0·3	= C.
(5)	1 „	+	0·08 „	+	0·3 „	+	0·1	+	0·4	= +++
(6)	1 „	+	0·08 „	+	0·3 „	+	0·08	+	0·42	= ++
(7)	1 „	+	0·08 „	+	0·3 „	+	0·06	+	0·44	= +?
(8)	1 „	+	0·08 „	+	0·3 „	+	0·03	+	0·47	= 0
(9)	1 „	+	0·08 „	+	0·3 „	+	0·01	+	0·49	= 0
(10)	1 „	+	0·08 „	+	0·3 „	+	0	+	0·5	= 0

1½ hour at 37° C.

(2) *With Excess of Heated Immune Serum.*

	1 per cent. O.B.C.		H.I.S. 1 in 10.		H.I.S.		Compt.		Saline.	
(1)	1 c.c.	+	0·08 c.c.	+	0·3 c.c.	+	0·5	+	0	= C.
(2)	1 „	+	0·08 „	+	0·3 „	+	0·4	+	0·1	= C.
(3)	1 „	+	0·08 „	+	0·3 „	+	0·3	+	0·2	= C.
(4)	1 „	+	0·08 „	+	0·3 „	+	0·2	+	0·3	= C.
(5)	1 „	+	0·08 „	+	0·3 „	+	0·1	+	0·4	= C
(6)	1 „	+	0·08 „	+	0·3 „	+	0·08	+	0·42	= C.
(7)	1 „	+	0·08 „	+	0·3 „	+	0·06	+	0·44	= C.
(8)	1 „	+	0·08 „	+	0·3 „	+	0·03	+	0·47	= C.
(9)	1 „	+	0·08 „	+	0·3 „	+	0·01	+	0·49	= +?
(10)	1 „	+	0·08 „	+	0·3 „	+	0	+	0·5	= 0

1½ hour at 37° C.

One per cent. ox blood corpuscles suspension was used; M.H.D. of heated immune serum used, for 1 c.c. 1 per cent. suspension of ox blood corpuscles = 0·08 of a 1 in 10 dilution. This dilution was used for sensitisation of the corpuscles; while the full strength serum in doses of 0·3 c.c. was added to give a great excess of immune body.

Here it will be seen that the addition of 0·3 c.c. heated immune serum (H.I.S.) lowered the M.H.D. of the complement from 0·2 c.c. of 0·03 c.c. The heated immune serum itself contained no complement, for 1 c.c. of it + 1 c.c. 1 per cent. suspension of ox blood corpuscles incubated at 37° C. for one and a half hour showed no hæmolysis.

These experiments may be divided into two sets—the first where sensitised, saturated, and non-sensitised corpuscles were injected into fresh rabbits, and the second where sensitised (saturated) and non-sensitised corpuscles were injected into immune rabbits. In the first set it will be seen that the injection of corpuscles, sensitised, saturated, or nonsensitised intravenously in sufficient quantity to use up practically all the complement in the animal's blood, if the animal were bled to death and the test made with the serum obtained *in vitro*, has produced absolutely no effect in the complement content of the serum, as shown by the M.H.D. of the complement before and after injection being the same. This has happened in spite of the fact that where sensitised and saturated corpuscles were injected, hæmolysis *in vivo* did take place, as shown by the intense hæmoglobinuria and the intense, deep red colour of the serum obtained after injection. Where non-sensitised corpuscles were used for injection no effect was produced, the urine not being tinted, and the serum was only coloured to an extremely small degree. This last might have arisen from hæmoglobin present in the serum injected due to the clot being manipulated to hasten separation.

The results obtained from the sensitised and saturated corpuscle injections may indicate that complement is not a definite limited quantity in the plasma of the blood, that it is not free in the plasma, but that it is given off from contact of its source with certain foreign bodies (in these experiments, the sensitised corpuscles injected), and that it is extremely rapidly regenerated. They seem to show that the tissues have a great reserve power for the production of complement, a condition apparently inconsistent with the view that the complement is a limited quantity free in the plasma. Complement may be present in the plasma, but there is no direct proof that it is, and the characteristics of complement action have only been definitely determined in serum *after clotting*. The introduction of a sensitised foreign body (*i.e.* possibility of starting the initial stages of clotting) into the blood stream has been shown above to bring about a phenomenon suggestive of complement action (namely, the hæmoglobin tinted serum and urine). In spite of this no fall in complement was

(as has been shown) detected between the bleedings. To account for the complement reading remaining the same, it seems necessary to suppose that fresh complement passed into the circulation during the experiment. Such considerations naturally raise the question of the existence of complement in a free state, and suggest a vital relation between a living cell and a foreign body.

The above experiments also show the necessity for the presence of both complement and immune body for hæmolysis *in vivo*; for when unsensitised corpuscles were injected into a fresh rabbit there was practically no hæmolysis to be noted; in later experiments, performed with special care to determine this point—especially to allow absolutely spontaneous separation of the clot from the serum, the serum after injection showed not the slightest trace of hæmolysis.

In the Series II., where sensitised (saturated) and non-sensitised corpuscles were injected into an immune rabbit, the effect is different; but here again a vital reaction of the tissues in the production of complement may be suggested. In all the cases where the corpuscles were injected into an immune rabbit the specific action of the complement was much less after injection than before injection, as shown by the same moiety of serum after injection producing much less hæmolysis *in vitro* than before injection. This lowering of the complement action has taken place in spite of the fact that the complementing power (not necessarily the *amount* of complement, *vide* p. 528) of an immune rabbit's serum is much greater than that of a fresh rabbit, and that consequently the injection of equal quantities of blood corpuscles into immune and fresh rabbits of the same blood volume should, instead of causing a diminution of complementing power in the immune rabbit, rather favour the complement remaining at what it was before injection. An example may make this clear. In Series II., Experiment 1, a fresh doe rabbit, weight 1460 grms., was injected with 2·8 c.c. saturated ox blood corpuscles. The complement remained the same before and after injection. Here, if we take 0·6 c.c. (see Experiment) as the M.H.D. for 2 c.c. 1 per cent. ox blood corpuscle suspension, then the 2·8 c.c. should have used up 84 c.c. of complement; but the total amount of complement present in the animal's body was only about 38·6 c.c.; yet in spite of this the complement content remained the same before and after injection. On the other hand, in Series IV., Experiment 1, where an immune buck rabbit, weight 2150 grms., was injected with 3 c.c. fully saturated ox blood corpuscles, the complement was very greatly diminished in the serum obtained after injection. Here, if we take 0·15 c.c. (see Experiment) as the M.H.D. of complement for 2 c.c. 1 per cent. suspension of ox blood corpuscles before injection, then the 3 c.c. ox blood corpuscles ought to have used up only 22·5 c.c. of the complement; while the amount of complement present in the whole animal's body amounted to 52 c.c. This might be explained by

supposing some change to occur in the vital production of complement in an immune rabbit as the result of its immunity, allowing complement to be more easily dissociated from the cells with less reserve power in the body for production of a large quantity of this substance; alternatively, the injection of the corpuscles might so derange the vital functions and upset metabolism that for the moment the organism does not respond, and the complement determination after injection would thus show a diminution. In support of this latter view is the fact that all the immune rabbits injected were severely ill, three of them practically dying before the expiry of the one and a half hour limit—one actually dying in five minutes. This may have been due to minute emboli of red cell stromata or agglutinated red cells; and evidence of infarction was seen in several of the rabbits; but there is a possibility of a breakdown of the whole mechanism of the body, comparable to the breakdown occurring in horses highly immunised against diphtheria-toxin on the injection of a further dose.[1] However the breakdown occurs, the fact that the animals were extremely ill shows that this might have something to do with the diminution of complement.

There are some facts which point, however, to there being a gradual change in complement production in immune rabbits as the result of the immunisation. Of the six fresh rabbits injected, only one showed any sign of illness. It was very ill for the first half-hour, and looked as if it would die. Here the cause of the illness was undoubtedly minute flocculi visible to the naked eye in the blood suspension used for injection. In spite of this illness there was no diminution of complement in the serum obtained after injection. Again, the immune rabbit which had been immunised for about six months (Experiment 1, Series V.), and which died in five minutes, showed of all the rabbits the greatest diminution in complement in the serum obtained after injection. This might have been due to lack of time for regeneration, but the marked diminution is significant, especially when taken in connection with rabbit of Experiment 2, Series V. This rabbit had only been immunised for about three weeks. After injection it was very ill, but recovered and looked quite like living when the time limit (one and a half hour) expired. Here the diminution in complement was very slight indeed in the serumof the injection. Whatever view is taken, as to the immediate cause after diminution of complement,—whether there is a so to speak momentary lowering of vital reaction caused by the flooding in of the corpuscles, or a more prolonged moulding action causing complement to be more easily dissociated and less easily regenerated, as in the case of the fresh rabbits,—the supposition of a vital reaction of some issue in relation to a foreign body causing that tissue to give off complement may be entertained.

[1] This point is being further investigated at present.

Conclusion.

The injection intravenously of unsensitised, sensitised, or saturated ox blood corpuscles into fresh rabbits produces no diminution of complement; whereas the injection intravenously of unsensitised or sensitised ox blood corpuscles into immune rabbits (*v.* ox blood corpuscles) produces a marked diminution in complement.

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REFERENCES.

Douglas, C. G. . . . . *Journ. Physiol.*, Cambridge and London, 1905–6, vol. xxxiii. p. 493.

Ehrlich and Morgenroth — In Ehrlich's "Collected Studies on Immunity," New York, 1906, p. 72.

PLATE I.

Figs. 1,2,& 3 show various stages in glycogenic functioning of the rabbits liver. Fig 3 being most advanced.

Fig.4. shows at least 3 hens' corpuscles inside a liver cell of a rabbit which ½ hour previously had been injected intravenously with 2cc washed hens' corpuscles.

Fig.5. shows the accumulation of hens' corpuscles in the liver of a rabbit which 1 hour previously, had been injected intravenously with 2cc washed hen corpuscles. This drawing from the liver is taken at a time when as far as could be seen, hen corpuscles had been absent for some time from the general circulation, and when only one or two corpuscles could be found in a whole section of the spleen and none in sections of the other organs.

PLATE II.

Fig. 1. from the liver of a rabbit. Showing the vacuoles in the liver cells.

PLATE III.

Figs/

Figs. 1 & 2. are from the liver of Dr.Stuart McDonald's primipara case. Fig.1 shows vacuolations of the liver cells, one of the vacuoles containing a red blood corpuscle, and some of the other vacuoles containing brown pigment. Fig.2 shows an advanced stage of the vacuolation of the liver cells.

Plate I

fig. 1

fig. 2

fig 3

fig 4

fig 5.

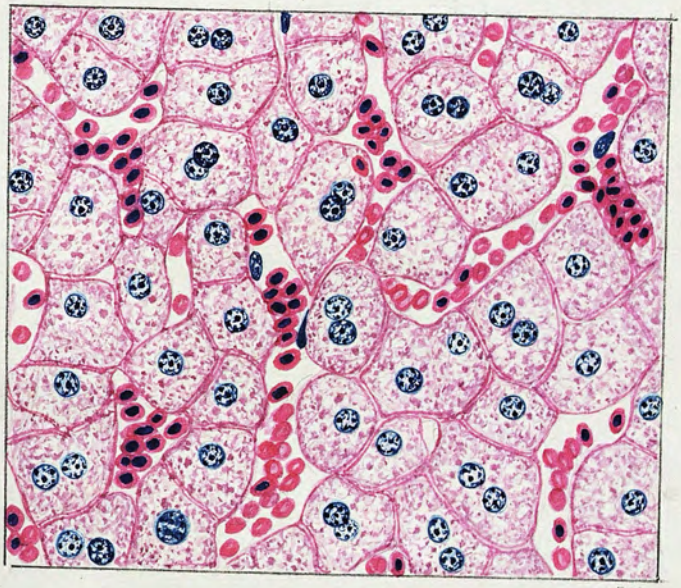

Plate II

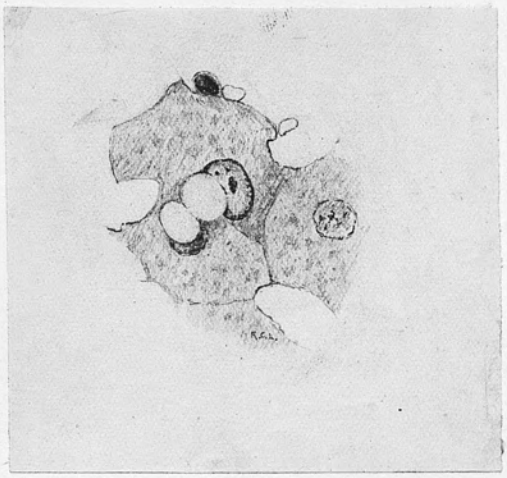

fig 1.

Plate III

fig. 1

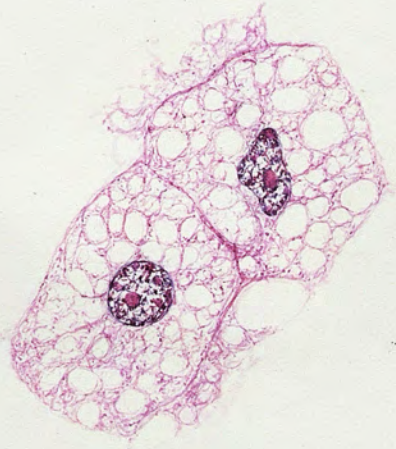

fig 2.